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LEVODOPA IN TREATMENT OF DECOMPRESSION SICKNESS AND OF AIR EMBO--ETC(U)
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FINAL TECHNICAL REPORT

LEVODOPA IN TREATMENT OF DECOMPRESSION SICKNESS AND OF AIR EMBOLISM INDUCED PARAPLEGIA IN RATS

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TABLE OF CONTENTS

ABSTRACT

The main objective of this project is a study of the protective effects and mechanisms of levodopa and some other agents in the prevention and in the recovery of rats from decompression sickness. For better clarity the description of performed work is divided into two parts -- air embolism studies and decompression sickness studies.

A. Air Embolism Studies. We have shown that rats made paraplegic by air embolization recover better after levodopa administration than control paraplegic animals. In order to ascertain that beneficial effects of levodopa is not species specific as well as that it is applicable in the treatment of paraplegias of other origins, rabbits were used and paraplegia was induced by a 45 min occlusion of the abdominal aorta. Control rabbits did not recover from the induced paraplegia, while 10 mg/kg levodopa led to full or partial recovery of the animals after 1-4 days. We have reported that levodopa has an extremely prolonged cardiovascular and pressor effect when administered to hypothermic rats. We have shown that dopamine administration of ten percent glycerol significantly improves the survival of rats after cerebral infarction. Simultaneous administration of levodopa and glycerol significantly improved the survival of infarcted animals. It appears, therefore, that control of edema should be the primary step in therapy after cerebral infarction or after embolization of the brain occurring in decompression sickness.

B. Decompression Sickness Studies. We have shown that gelatin, an agent that protects platelets during freezing and thawing, protects rats from DS. The difference between the control and the gelatin treated groups was highly significant. Saline pretreated animals fared somewhat better

than the animals in the control group, but the difference in results between the two groups was not statistically significant. The beneficial effect of gelatin may be attributed to the coating of circulating platelets and small blood vessels or to the coating of air bubbles during development of DS. Pretreatment with gelatin and levodopa (given 30 min before compression-decompression) seems to be beneficial for faster recovery from DS, though it did not decrease the number of animals that showed clinical symptoms of DS. Using Murphy's multiple hit model for computing platelet survival that permits fitting of any one of a family of gamma functions to the raw platelet (^{51}CR) survival data, we observed a profound decrease of mean platelet survival from 4.02 days to 2.37 days and a decrease in mean recovery from 74% to 46% after exposure of rats to compression-decompression profiles. Pretreatment with gelatin returned the values toward the control level. Aspirin pretreatment (40 mg in drinking water for 30 days) was highly beneficial in preventing occurrence of DS. Only 12 per cent of the animals died after the dysbaric exposure as compared to 31 per cent in the control group. Pretreatment with heparin, however, was not beneficial. Pretreatment with levodopa and aspirin was beneficial in protecting rats from DS (6 per cent mortality), more than the use of any other agent. Though we have at the present only 40 animals per group, the mortality rate of rats in DS decreased from 33% to 4% after prolonged pretreatment with levodopa. Thus combination of pretreatment with levodopa (4 days) and aspirin (30 days) or prolonged pretreatment with levodopa (30 days) were the most beneficial in decreasing incidence of DS in rats after compression-decompression exposure.

INTRODUCTION

The objectives of this work were to study protective effects and mechanisms of action of levodopa, glycerol and other agents in the prevention and recovery from induced paralysis and in prevention and recovery from experimentally induced decompression sickness. To induce paralysis, various methods were employed, as for instance intravascular injections of air, clamping the abdominal aorta and administration of carbon microspheres. Decompression sickness was induced after a sudden compression-decompression. Rats and rabbits were used in the experiments. For better clarity, the work done during the last 5 years is divided into two parts: a) air or microsphere embolization studies, and b) experimental decompression studies.

This report summarizes the work performed during this contract by providing a brief introduction to the various experiments followed by the pertinent data. At the end of the report (in the form of Addenda), published personal work and manuscripts in publication are added.

A. Air Embolization, Occlusion of Aorta and Microsphere Infarction Studies

1. Levodopa effects on recovery from air embolism induced paraplegia. In this study, levodopa was chosen as an agent that might ameliorate clinical manifestations of paralysis induced by air embolism because of known effects of this drug on muscle spasticity and locomotion. In order to induce air embolism, the aorta of rats was cannulated chronically (Popovic and Popovic, J. Appl. Physiol. 1960). Fifteen days later, after full recovery of the animals, air was administered through the chronic cannula into the descending aorta of each rat (0.35 ml of air per 100 g during 4 seconds). Paralysis of both hind legs was observed one to ten minutes later. Only animals that had total paralysis of both hind legs, with no sensation, were used in the experiments. Levodopa was administered 2 minutes after experimentally induced paralysis. The levodopa treatment was repeated every day for 6 days. Eighty percent of levodopa-treated (intraperitoneally or intra-arterially) animals recovered fully from the paralysis. Only twenty percent of untreated animals or animals that were injected with solvent recovered without the levodopa treatment. The difference in recovery was highly significant. The results of this work were published and are attached to this final report as Addendum I (Popovic, Pava, V. Popovic and R. Schaffer. Surgery 79, 100-103, 1976) and Addendum II (Popovic, Pava, V. Popovic and R. Schaffer. Aviat. Space Environm. Med. 1073-1075, 1976).

2. Levodopa in recovery of rabbits from paraplegia induced after occlusion of abdominal aorta. Occlusion of abdominal aorta for 45 minutes leads to paraplegia in rabbits. The duration of occlusion needed to develop paraplegia was established by recording the moment of disappearance of all evoked potentials in the brain. Control rabbits did not recover from paraplegia. Levodopa (10 mg/kg administered through ear vein) led to partial

or full recovery in all treated rabbits.

3. Effect of levodopa on arterial blood pressure in unanesthetized rats. As described above, the beneficial effect of levodopa in recovery from air embolization was established in unanesthetized rats. However, until now the cardiovascular effects of levodopa were studied in anesthetized animals only. Therefore, in this work the effects of levodopa on mean arterial blood pressure were studied in unanesthetized rats and compared with the results obtained after the animals were anesthetized with nembutal (i.p., 40 mg/kg) or with halothane. The arterial blood pressure was recorded in rats with chronically implanted aortic cannulas after full recovery from anesthesia and surgery (at least 15 days). Levodopa (10 or 20 mg/kg, i.a.) was administered 10 minutes after the animals reached the desired level of anesthesia. After levodopa administration, the mean arterial blood pressure of all rats was increased, reaching the peak value 2-5 minutes later. The animals in halothane anesthesia had a small rise of the mean arterial blood pressure. Doubling the dose of levodopa increased further the hypertensive responses in unanesthetized animals but not in the animals under nembutal anesthesia. Levodopa causes a much greater increase (percent) of the mean arterial blood pressure in nembutal groups than in unanesthetized animals. The results of this work were published and are attached to this report as Addendum III (Popovic, Pava, V. Popovic, R. Schaffer and A. S. McKinney. Proc. Soc. Exp. Biol. Med. 154, 391-396, 1977).

4. Treatment of experimental infarction in rats with levodopa or glycerol. Levodopa is a monamine which rapidly passes the bloodstream barrier, and glycerol is an agent that reduces brain edema due to its hyperosmolarity. In order to establish whether cerebral infarction with air (or with carbon microspheres) can be treated with levodopa and/or with glycerol, the following

experiments were done.

Induction of cerebral embolization in female Sprague-Dawley rats was done with carbon microspheres through a polyethylene cannula (PE 50).

The results of this study indicate that levodopa did not improve the survival of rats after acute cerebral infarction induced by injection of carbon microspheres, but when 10% glycerol was used, the survival (after cerebral infarction) was significantly greater than in the control or in the levodopa-treated rats. Simultaneous administration of levodopa and glycerol also significantly improved the survival of infarcted animals. This result suggests that glycerol alone is the main factor in eliciting this beneficial effect. Therefore, it appears that control of edema should be the primary step in therapy after acute cerebral infarction or acute cerebral air embolization.

Table 1.

Survival of rats after controlled cerebral infarction (microspheres) treated with levodopa and/or glycerol

	No. of	Survival Day							Survival
	Animals	1	2	3	4	5	6	7	Animals No.
Saline	22	19	9	5	5	5	5	5	5 (22)
Levodopa (Repeated)	10	9	5	4	3	3	3	3	3 (10)
Glycerol (Repeated)	22	19	14	14	14	14	14	14	14 (22)
Glycerol + Levodopa (Repeated)	10	9	6	6	6	6	6	6	6 (10)

The results of this study were published and are attached to this report as Addendum IV (Popovic, Pava, V. Popovic, R. Schaffer and C. H. Sutton. J. Neurosurg 48, 962-969, 2978).

5. Histologic study of infarcted brains or rats treated with levodopa and/or glycerol. Glycerol-treated female rats have a decreased mortality after glycerol administration. This is probably because of glycerol's hyperosmolitic property since pathological findings demonstrated that repeated treatment with glycerol produced a marked decrease in brain edema, confining the edema to focal areas adjacent to microspheres. Infarction resulting in cavity formation was not prevented, but edema and cellular reaction around these zones of infarction were markedly reduced in size and sharply circumscribed. Pathological changes of brain tissue in animals treated with levodopa and glycerol were similar to those found in only glycerol-treated animals. Animals in the control group had generalized edema of the ipsilateral hemisphere, as well as short survival time. It appears, therefore, that the use of glycerol treatment immediately -- and repeatedly -- after experimental infarction with carbon microspheres reduces the extent of brain edema and cellular reaction. Furthermore, treatment with levodopa and glycerol was as effective as with glycerol alone, indicating that in future experiments this combination of agents might be useful in DS rat experiments.

6. Hypothermia as dopamine agonist. Cardiovascular changes that occur after administration of levodopa or of dopamine are very profound. Besides cardiovascular effects, other physiological effects have been described after levodopa administration. Levodopa is a precursor of dopamine and or nonrepinephrine, neurotransmitters that regulate arterial blood pressure and heart action. Levodopa is a drug used for successful treatment of Parkinsonism. Dopamine stimulates myocardial beta-adrenergic receptors, but in the smooth muscles of systematic vascular bed dopamine acts either on alpha-adrenergic receptors or possibly on specific dopamine receptors. Beneficial effects of lovodopa and dopamine have also been demonstrated in

the treatment of cardiogenic shock. Levodopa induces mesenteric, renal and cerebral vasodilation.

In this work we studied the effect of hypothermia on pressor responses to administered levodopa. It is known that in profound hypothermia, many physiological processes slow down and some of them cease temporarily. It is unknown whether or how much the action of dopamine on its receptors is changed when the temperature of the receptors is decreased. In other words, we tried to ascertain whether the adrenergic receptors retain their characteristic responses at a low body temperature. Levodopa or norepinephrine were used in doses that produce a large hypertensive response in the normothermic animals. Chronically cannulated adult female Sprague-Dawley rats, weighing 200 $\pm$ 10 g, were used. During blood pressure measurements, each animal was placed in a transparent plastic box (4 x 10 x 4"). The exterior part of the chronic cannula was connected by a needle adapter (30 gauge) to a pressure transducer (P 23 De, Statham) and a polygraph (Beckman). The arterial pressure of the animal was recorded only during the complete resting state of the animal. An opening in the middle of the box cover permitted connection of the chronically implanted cannula to the transducer and free movement of the animal during pressure measurements. The duration of blood pressure measurement varied with the experimental procedure and lasted from 1 to 4 hours. Levodopa (Hoffman LaRoche) was dissolved in warm saline and was administered through the chronic cannula (20 mg/kg); the volume never exceeding 0.1 ml. Norepinephrine (0.1 mg/kg) and phenoxybenzamine were injected through the chronic cannula. The arterial blood pressure measurements were always made at the same time of the day. The colonic temperature was monitored (Honeywell recorder and thermocouples). Hypothermia (17^{o}-18^{o}C) was induced by hypoxic-hypercapnic cooling technique and the cooling (Popovic and Popovic. "Hypothermia in Biology and in Medicine."

Grune and Straton, pp. 345, 1974) was initiated with light anesthesia. After the experiment, each animal was rewarmed to the normal temperature by infrared lamp. The mean arterial blood pressure of hyperthermic rats (body temperature 17°-18°C) was 82 ± 11 mmHg. After administration of norepinephrine, the pressure increased to 138 ± 12 mmHg but returned to the normal low value after 15 minutes. The administration of levodopa increased the mean arterial blood pressure from 87 ± 7 mmHg. The pressure stayed at this level (without decreasing to the low level) for 4 hours. (Fig 1).

Thus it appears that low body temperature acts as a hypertensive dopamine agonist, a fact that might explain the beneficial effect of levodopa combined with hypothermia in the recovery from experimentally induced paraplegia.

This work has been reported recently at the scientific meetings (Addendum V). Manuscript of a full paper has been submitted.

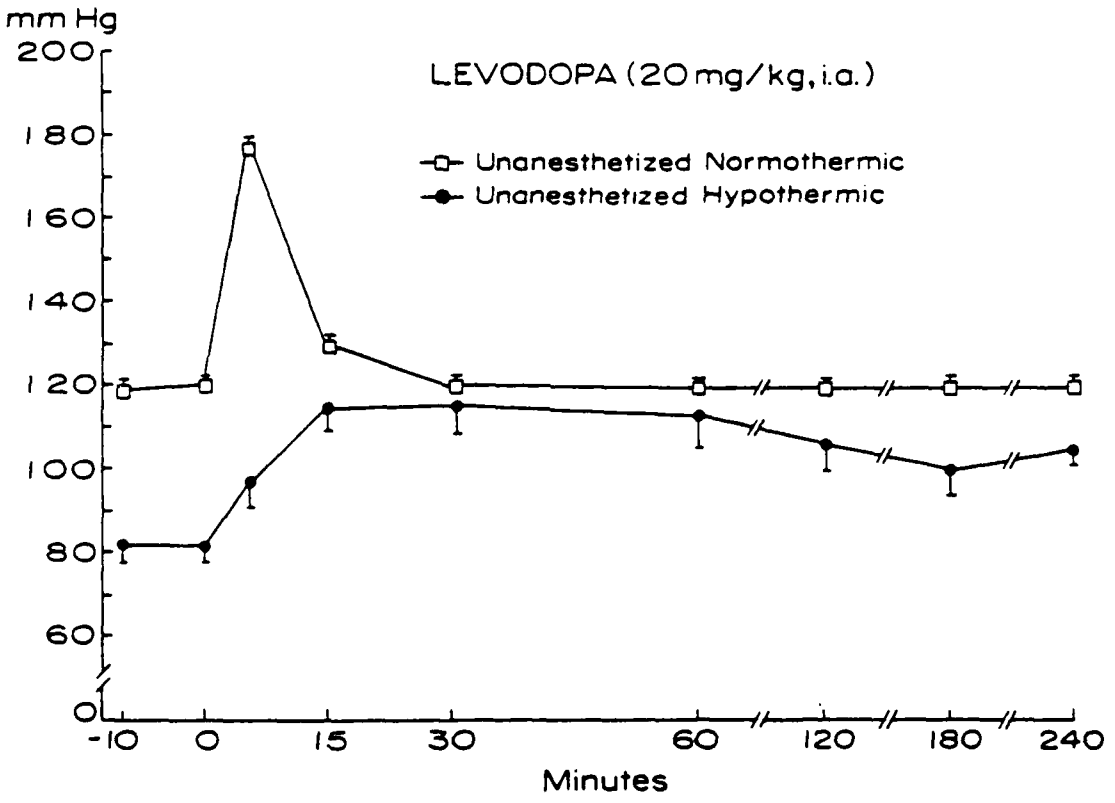

Fig. 1. Hypertensive responses of levodopa in normothermic and in hypothermic rats.

7. Mean arterial blood pressure of hypothermic animals during rewarming after administration of levodopa. After the mean arterial blood pressure was measured, the levodopa was administered to hypothermic rats. Infrared rewarming was initiated once the maximal pressure response was reached (after 30 min), and the animal warmed until the normal body temperature was reached. The arterial blood pressure stayed at the peak level until a body temperature of 21°C was attained, then the arterial blood pressure suddenly decreased to the control (hypothermic) level. It should be noted that, at 21°C, during rewarming, the thermoregulatory mechanisms reappear. Thus, with the onset of shivering, the levodopa induced long-lasting hypertensive response is suddenly terminated. After 21°C, during further rewarming, the arterial blood pressure followed the same curve as control hypothermic animals during rewarming.

8. Mean arterial blood pressure in rats treated with phenoxybenzamine after levodopa administration. The administration of this drug to normothermic or to hypothermic animals blocked the hypertensive response induced by levodopa.

In conclusion (chapt. 6-8), it appears that levodopa acts on alpha-adrenergic receptors in hypothermic animals as well. Furthermore, when levodopa is administered to hypothermic rats, the induced hypertensive response is of a very long duration. It is possible that this hypothermic potentiation of the cardiovascular effects of levodopa is the main cause of the beneficial effects of levodopa on recovery from paraplegia since this effect is observed only in spontaneously hypothermic animals. The cardiovascular effect of norepinephrine in hypothermic rats is similar to that of normothermic animals. Inactivation of some of the enzymes that permit formation of norepinephrine from its precursor seems to be the likely cause for this strikingly different cardiovascular effect of levodopa in hypothermia.

9. BCNU toxicity in systemic versus internal carotid administration.

Rodents have proven to be good subjects for evaluating biological effects of chemotherapeutic drugs. In this study, we used the rat, an animal which is sufficiently large for hematologic studies and one which is often used in brain tumor research. Furthermore, the aorta of the test rats was chronically cannulated for BCNU administration and for repeated blood sampling in unanesthetized and unrestrained conditions.

The treatment of malignant gliomas and of other brain tumors derived from systemic cancers is not fully successful. The introduction of nitrosoureas (BCNU, CCNU) made additional progress in the treatment of brain tumors. A lipid soluble agent, 1,3-bis (2-Chloroethyl)-1-Nitrosourea (BCNU), appears to enter and distribute to the same extent both in brain tumors and in the normal brain tissue. This is of importance because most brain tumors are infiltrative in character. Malignant cells infiltrate normal brain tissue on the periphery of the tumor mass. The BCNU is an alkylating drug, and in order to be therapeutically effective, it must be used in concentrations very near to the toxic levels. In order to increase the concentration of the drug in the brain tissue, in our experiments BCNU was administered into the internal carotid artery. It was speculated that such a therapy would be beneficial by decreasing systemic toxicity of BCNU, while at the same time increasing and localizing the effect on the target area. To better evaluate the effect of internal carotid administration of BCNU, the drug was also administered systemically.

Female Sprague Dawley rats, weighing 212 ± 21 gm (SD), and kept in individual cages, were used in the experiments. Under fluothane anesthesia, a polythylene cannual (PE 10) was inserted (for chronic use) through the left common carotid artery into the aortic arch of all rats.

The peripheral end of the cannula was exteriorized on the back of the animal's neck. Besides chronic cannula, an acutely placed PE 10 cannula in the internal carotid was used for BCNU administration into the brain. This cannula was removed immediately after BCNU injection. Only the animals that had a normal total WBC ten days after recovery from surgery were used in the experiments. The animals received 10 mg/kg (0.68 ml) BCNU either into the internal carotid artery or systemically. The control animals did not receive any drug. Another group of animals received ethanol (0.6 ml of 10% solution). The arterial blood was sampled during the following seven days. At the end of the seventh day, the animals were sacrificed and brain, hepatic, and renal tissues were submitted for histological evaluation. In the control group of animals, the total WBC (followed daily during 7 days) stayed in the normal range for this species. Histologic findings demonstrated liver, kidney, and brain tissue to be normal. In animals after administration of ethanol into the internal carotid artery one day after injection of ethanol, the total WBC was decreased. The return to the initial value occurred on day two. The histologic findings were similar to the control animals. In animals after systemic administration of BCNU, the nadir of the total WBC was on day two, and the return to initial value was on day seven. There was a statistically significant difference between the animals in this group and animals in the control group. Pathologic findings have shown that hepatic tissue was normal in all animals. Four of seven animals had mild to large areas of subcapsular renal necrosis. Brain tissue of all animals in this group had no significant inflammation, edema or necrosis. In animals after administration of BCNU into the internal carotid artery, one observed a decrease in the total WBC on day two. The return to the initial

value occurred on day six. A statistically significant difference in the total WBC existed on day two between the animals in this group and in the control group. No significant difference was found between the systemic BCNU group and the internal carotid BCNU group. Pathologic findings demonstrated that animals had normal hepatic tissue. Two animals had small subcapsular necrotic foci in the kidney tissue. Five of the seven animals had changes in brain tissue in both hemispheres consisting of necrosis and reactive histiocytic infiltrates.

It has been reported that BCNU is more effective than other anticancer drugs in the treatment of experimental animal gliomas and in patients with brain tumors, especially when combined with surgery and irradiation. In our work we used chronically cannulated rats to study the effects of BCNU. The chronic cannulas enabled the exact doses of the drug to be administered into the blood stream of unanesthetized animals and allowed daily blood sampling throughout an extended period. The delivery of drugs to the brain or to a brain tumor depends on the blood-brain barrier as well as on the route of drug administration and on the metabolic activity of the drug. It was speculated that BCNU administration through the internal carotid pathway would be beneficial in brain tumor therapy due to an increased local drug concentration and to a decreased systemic toxicity. However, the internal carotid administration of a drug is a more difficult procedure and might increase the possibility of intracarotid clotting. Only a few clinical cases have been reported in which intra-arterial treatment was used. It is difficult, therefore, to evaluate the real benefit of such a treatment at the present time.

The results indicate that there is a statistically significant difference in total WBC on day two between both groups of BCNU-treated rats and control rats. After systemic administration of BCNU, the rats

had damaged kidneys while the brain was hardly affected. Contrary to this, after internal carotid administration the induced damage was in both brain hemispheres while the kidneys were spared. The liver was not affected in either situation. Examination of the specimens in our study did not demonstrate any unequivocal thrombosis in the small vessels of the brain which we could interpret as being caused by the administration of the BCNU. A few vessels could be shown to contain agglutinated erythrocytes. This was interpreted as a post-mortem change. In conclusion, the internal carotid administration of the BCNU increased the effectiveness of the drug on the brain tissue and decreased the drug's systemic toxicity. (The results of this work have been submitted to Neurooncology journal, Addendum VI).

B. Studies of Decompression Sickness in Rats

1. Compression-decompression profiles. A Vacudyne Animal Hyperbaric Chamber with a maximum pressure of 100 psi at 100°F was used in our work. A wooden base of 18 x 53 inches carries two plexiglass rat cage units (12 x 40 x 6 inches). Each unit has 10 perforated individual animal cages (5 x 7 x 5 inches). The chamber has four large ports, two for viewing animal activities and two for leads for recording temperature, blood pressure, ECG, etc. A maintenance flow valve has been adapted so that samples of chamber gases can be obtained. The temperature in the chamber is regulated. Special adaptation was installed for noise elimination during decompression.

2. Procedure for inducement of decompression sickness in rats. Animals. Adult, female Sprague-Dawley rats weighing 200 ± 20 g are used in the experiments. All animals are housed individually and given food and water ad libitum. Two to three weeks before compression-decompression, each animal is anesthetized with Fluothane anesthesia. A polyethylene cannula (PE 10) is permanently implanted in the left carotid artery with the tip floating in the aortic arch and its free end exteriorized on the back neck of the animal (Popovic and Popovic, J. Appl. Physiol. 1960). The cannulas remain open for the lifespan of the animals (Popovic et al., Proc. Soc. Exp. Biol. Med., 1963).

Before a decompression experiment, all food is removed from the cages. After 18 hrs, the fasted, unanesthetized animals are placed in individual, perforated plexiglass cages of the plexiglass cage unit. The unit is placed in the hyperbaric chamber and the chamber prepared for a pressurization-compression-decompression (diving) regime modified after Sallee (Sallee and Adams, Aerospace Med., 1970).

Pressurization. The Vacudyne Animal Hyperbaric Chamber containing the animals is initially pressurized to 30 psi (compressed air) at a rate of 6 psi/min. At the chamber pressure of 30 psi the compressed air is changed to a different compressed gas mixture (95% nitrogen and 5% oxygen) for pressurization to 100 psi at a rate of 10 psi/min. Elapsed time is 5 min for pressurization from 0-30 psi and 7 min for pressurization from 30-100 psi, for a total of 12 min.

Compression. Upon reaching a chamber pressure of 100 psi, a ventilation rate of 10 liters/min with a driving pressure of 120 psi is established throughout the duration of the compression period.

Decompression. Decompression begins after ventilation is interrupted and the decompression valve is opened. In order to achieve chamber decompression within 2 min, a decompression rate of 10 psi/15 sec is maintained from 100 psi to 30 psi. This requires 1 min 45 sec. At 30 psi a ball valve is opened permitting a rapid decompression from 30 psi to 0 psi within the final 15 sec (total time 2 min). Upon reaching 0 psi the chamber door is opened and the cage unit containing the rats is removed.

Administration of drugs. The drugs are administered and blood is sampled through permanently implanted aortic cannula.

Scoring index. The following index was used: 0. no symptoms; 1. labored breathing; 2. paresis; 3. paralysis; 4. death. Time for each symptom to develop is recorded as well.

Results obtained after compression-decompression of rats. The chronically cannulated rats were simultaneously exposed to the same compression (30 min or 60 min) -decompression profile as uncannulated animals of the same size, weight, sex, age, etc. There was no difference in obtained results between these two groups.

3. Gelatin pretreatment and experimental decompression sickness.

Processes and mechanisms that lead to decompression sickness (DS) are not well understood. Until recently it was thought that the mechanical action of air bubbles was the main cause of DS. However, new data indicate that the process is more complex and that many pathophysiological changes occur during rapid decompression. Surface activity at the blood-gas interface (Elliott et al., 1974) and intravascular bubble-platelet interaction (Philp, 1974) have been proposed as principal factors in the development of DS. The relationship between the amount of air bubbles in the blood and the severity of DS is still not well understood. For instance, ultrasonic devices occasionally detect intravascular bubbles in divers with no apparent clinical manifestation of DS (Evans et al., 1972). The thrombocytopenia that follows platelet involvement was observed as early as one hour (Broussolle et al., 1973; Giry et al., 1977) but also a full twenty-four hours after the dive (Martin et al., 1973).

Presently, rapid recompression is the primary therapy for DS. Other treatments are induction of hypervolemia (Lamy and Hanquet, 1973), use of hypothermia (Thienprasit et al., 1975), and use of drugs. Drug therapy after DS has not been successful, although some improvement has been described after heparin administration (Reeves and Workman, 1971; Bennet, 1972; Cockett et al., 1972; Saumerez et al., 1973). It has been reported that pretreatment with cyclohexanone HCP decreases incidence of DS in mice (Chrysanthou et al., 1971).

In this work we studied the effect of gelatin pretreatment on the development of clinical symptoms after rapid compression-decompression. We chose gelatin because granulocytes, extremely fragile white blood cells that are easily damaged during freezing, survive freezing-thawing and

actively phagocytize when protected with gelatin (Popovic et al., Cryobiology, 1977 a,b). It was speculated, therefore, that gelatin might also protect platelets from injury incurred in DS since intravascular activation and an increased utilization of platelets are probably major causes of DS. In order to evaluate the effect of hypervolemia *per se* as an ameliorating factor in the onset of DS, another group of rats received the same volume of saline as the experimental animals.

One hundred and eight Sprague-Dawley female adult rats were compressed to 100 psi (5% O_2, 95% N_2) for 30 min and decompressed within 2 min. After a 30 min dive in a group of 36 control animals, 19 developed decompression sickness (8 rats died). The experimental animals were pretreated with a single intra-arterial injection of 3% gelatin (25 ml/kg body weight). In this group of 36 rats, only 3 showed clinical symptoms of DS (1 rat died). The difference between the control and the gelatin-pretreated groups was highly significant ($P > .01$). Saline-pretreated animals fared somewhat better than the animals in the control group, but the difference between the two groups was not statistically significant.

Table 2.

Clinical manifestations of decompression sickness after a 30 min dive to 100 psi in control rats, in saline pretreated rats and in gelatin pretreated rats. All animals were grouped according to the most severe of DS symptoms.

			Animals with DS Symptoms			
Group	No. Rats	Body Weight, g	Labored Breathing	Paraplegia	Total paralysis followed by death	Animals with DS,%
Control	36	186 ± 8	6	3	8	48
Saline Pretreatment	36	184 ± 11	6	0	5	28
Gelatin Pretreatment	36	185 ± 9	2	0	1	8

The results of this work are being published at the present time. The manuscript of the work is submitted here as Addendum VII.

4. Recovery and survival of platelets and experimental decompression sickness. Protective effects of gelatin pretreatment. Surface activity of blood-gas interface (Elliott et al., 1974) and intravascular bubble-platelet interaction (Philp, 1974) have been proposed as principal factors in the development of DS. Our interest in platelets led us to develop several techniques to study in our rat model, the survival and recovery of ^{51}Cr-labelled platelets in vivo. In this work, we have studied the recovery and survival of ^{51}Cr-labelled platelets after exposure to compression-decompression and changes that are brought by pretreatment of the animals with gelatin. Platelets from donor rats were labelled according to the technique of Harker (J. Lab. Clin. Med. 77, 247, 1971). The blood was obtained from the aorta of donor rats under Halothane anesthesia. Nine ml of blood were diluted with 1 ml of ACD formula A. The blood was centrifuged for 10 min at 1500 rpm at room temperature in an International Centrifuge. The platelet-rich plasma was removed and the pH adjusted to 6.5 with 0.15 molar citric acid. The acidified platelet-rich plasma was respun at 2500 rpm for 15 min. The platelets were resuspended in a small volume of plasma and 100 µCi of ^{51}Cr (as $Na_2\ CrO_4$) was added. The incubation period lasted for 15 min at room temperature. Two-thirds of the platelet poor plasma was then added to the incubation mixture and the platelet-rich plasma was spun at 2500 rpm for 15 min. The supernatant was decanted and the platelet button washed with a small amount of platelet-poor plasma. After the washing, the button was resuspended in 12 ml of platelet-poor plasma. The button contains the platelets obtained from 4 donor rats, enough to do survival

studies on 6 recipient rats. The resuspended labelled platelets were placed in sterile tubes and centrifuged at 200xG for 5 min to remove residual red cells. The labelled platelets were injected into the tail vein of recipient rats. The sampling of 0.01 ml blood from recipient rats was done before compression-decompression, one hour, six hours, and twice daily for 5 days after decompression. The platelet survival after compression-decompression was determined from the disappearance of radioactivity from blood sampled at intervals after injection of ^{51}Cr-labelled platelets (Aster. Blood 26, 732, 1965). Survivals were calculated according to the multiple-hit model of E. A. Murphy (J. Chron. Dis. 26, 797, 1973). The entire computation is carried out on a PDP-10 computer. Recovery of injected platelets was calculated by multiplying the blood volume of the rat by the platelet activity as a percentage of the injected dose extrapolated back to time zero. Removal of 0.2 ml of blood per day does not change circulatory or hematologic characteristics of rats (Popovic and Kent, Amer. J. Physiol. 207, 767, 1964).

The results (47 rats) indicate that platelet survival after compression-decompression was much decreased but that pretreatment with gelatin brought the survival time back close to the normal range. The recovery of injected platelets was similar to the normal survival range when the animals were pretreated with gelatin but much decreased in non-pretreated animals.

5. Water deprivation in experimental decompression sickness.

A new approach to the search for mechanisms that cause decompression sickness is to study surface activity of the blood/gas interfaces and intravascular bubble-platelet interactions. There are indications that hypervolemia might be beneficial in preventing DS. Hypovolemia, on the

other hand, should increase the number and severity of the symptoms in a group of rats that are developing decompression sickness. In this work we studied the effects of 24 hour water deprivation on the development of DS. Since hemoconcentration and increased platelet production occur during experimental DS, we wanted to see if water-deprived rats have a higher incidence of DS. The PE 10 cannula, chronically implanted in the aorta, was used for sampling blood. Thirty Sprague-Dawley female rats weighing 200 ± 20 gm were used in this study. When compressed to 100 psi (5% O_2, 95% N_2) for 30 min and then decompressed rapidly during a 2 min period, the rats developed DS. The results showed that water deprivation led to a higher hematocrit ratio after decompression than that observed in control-compressed rats. Surprisingly, the water-deprived animals had significantly smaller percentage of DS, and fewer animals in this group died.

6. Platelet aggregation in the pathogenesis of DS. Effect of aspirin. Platelet aggregation might be one of the crucial factors in the pathogenesis of DS. Of course, the process of thrombosis is initiated by the aggregation of platelets. This occurs as a result of stimulation by some external agent (for instance, ADP or collagen). The initial phase of aggregation leads to the platelet "release reaction" with secretion of ADP, serotonin, prostaglandin G_2, and thromboxane A_2. These factors produce further, and longer-lasting, aggregation and eventual activation of the coagulation system, producing a fibrin clot. Platelet aggregates may occur in the blood stream and act as microemboli. Experimental work in animals has shown that in vitro formed platelet aggregates may also produce myocardial necrosis, probably by occlusion of small arterioles (Haft et al., 1972; Haft and Fani, 1973). Platelet aggregation might

lead to transient cerebral ischemia (Gunning et al., 1964; Millikan, 1965; Moore and Hall, 1968) and cerebral infarction (Kuntz, 1969; Millikan, 1969). Platelets from patients with ischemic cerebrovascular disease show increased aggregability in vitro (Millar, 1965; Danta, 1970; Mundall et al., 1972; Kalendovsky et al., 1975) and platelet adhesiveness (Acheson et al., 1972; Boneu et al., 1972; Bansal, 1978). However, there are reports that platelet aggregability is not changed in cerebrovascular disease (Ettinger et al., 1969; Gaston et al., 1971). Patients with transient ischemic attacks or strokes have an increased number of platelet aggregates (Wu and Hoak, 1975).

Attempts to modify platelet aggregation and prevent thromboembolic episodes by use of pharmacologic agents is an exciting and promising area of research. Intensive pharmacologic investigations are being carried out in vitro to discover new agents capable of inhibiting thrombosis. Of course, agents that inhibit platelet aggregation in vitro must be shown to have in vivo activity.

Aspirin, and some other drugs that decrease platelet aggregation, are used for patients at risk for recurrent cerebral ischemia (Fields and Hass, 1971; Didisheim et al., 1974; Yatsu, 1977), and, therefore, might be beneficial in DS as well. Aspirin inhibits platelet aggregation and secretion by preventing synthesis of prostaglandins (Weiss, 1976 and 1978) and thromboxane A_2 (Samuelson, 1977). Three to six hundred mg of aspirin administered to a human inhibits aggregation during the lifespan of a platelet (9.5 days). Although the inhibitor effects of aspirin on platelet aggregation can be demonstrated by in vitro experiments, aspirin did not show protection against arachidonate-induced cerebrovascular occlusion in the rat (Furlow and Bass, 1976) or during development of DS in man (Philp and co. Undersea Med. Soc. 6, 127, 1979) and in rats (Bennett and Brock.

Aerospace Med. 40, 607, 1969).

For this reason we have undertaken the following group of experiments. Aspirin (40 mg in drinking water, average daily amount per rat) was given to the animals for 30 days. Then the rats were exposed to the usual compression-decompression profile. Twenty-two percent of the rats in this group developed DS after the dive. The mortality rate was 12%. Death occurred 9.5 ± 4.6 min after the decompression. A significant difference in mortality rate existed between this and the control group (48% DS and 28% mortality).

The results of this work are accepted and being published in the Proc. Soc. Exp. Biol. Med. (Addendum VIII).

7. Levodopa pretreatment and experimental decompression sickness. Search for a drug that might be used in the effective treatment of DS has not been successful. Furthermore, because DS develops adruptly, such a drug would be of little value if vital organs are damaged. However, a drug that fails in treatment of acute DS may still be successful if used as pretreatment. For this reason our interest was focused on agents that might be effective in pretreatment, decreasing occurrence, severity, and mortality of experimentally induced DS. The blood-bubble interface in DS triggers platelet aggregation and damage to the vascular walls. The platelets seem to play a key role in the production of endothelial surface lesions. Furthermore, dead platelets increase hazards of coagulation in already hemoconcentrated blood of DS victims. For this reason aspirin was chosen see under B 6) and used in this work. Levodopa was used because it is beneficial in Parkinsonism, in decreasing rigidity, in increasing locomotion, and as a potent vasoactive drug. Levodopa appears to be helpful in recovery from experimental spinal cord injury, and in recovery from air embolism

induced paraplegia in rats (Popovic et al, 1976).

Twenty-eight percent of the animals in this group developed DS (Table 2), while the mortality rate was 16% (Table 3). Death of the animals was delayed to 21.2 ± 14.2 min after the decompression. There was a significant difference in mortality rate between this and the control group.

The results of this work are accepted and being published in the Proc. Soc. Exp. Biol Med. (Addendum VIII).

8. Pretreatment with levodopa and aspirin and decompression sickness. After combination pretreatment eleven percent of the animals developed DS (Table 3). The mortality rate in this group was only 5.6% (Table 3). There was a significant difference in mortality rate between this and the control group. The onset of death was delayed to 15.2 ± 6.7 min.

Chi-square and P values obtained from the contingency tables indicate a) a highly significant difference ($P < .01$) of mortality rates between untreated control rats versus levodopa pretreated animals, b) a highly significant difference ($P < .01$) between untreated control rats versus aspirin pretreated animals, c) a highly significant ($P < .01$) between untreated control rats versus aspirin and levodopa pretreated rats, d) no significant difference between levodopa pretreated animals versus aspirin pretreated rats, e) significant difference ($P < .05$) between levodopa pretreated animals versus animals pretreated with aspirin and levodopa, and f) significant difference ($P < .05$) between aspirin pretreated rats versus animals pretreated with aspirin and levodopa (Table 4).

Table 3. Clinical manifestations of DS in rats after rapid decompression.

Group	No. rats	Body weight, g.	Animals with DS symptoms		Animals with DS, %
			Paraplegia	Total paralysis followed by death	
Control	48	202 16	4	15	40
Levodopa	50	212 16	6	8	28
Aspirin	50	214 14	5	6	22
Aspirin and Levodopa	54	212 15	3	3	11

Table 4. Composite statistical evaluation* of DS mortality in rats after pretreatment with levodopa and aspirin.

Animal Group	Mortality Rate	Level of Significance
Control	31.3%	<.01
Levodopa	16.0%	
Control	31.3%	<.01
Aspirin	12.0%	
Control	31.3%	<.01
Aspirin and Levodopa	5.6%	
Levodopa	16.0%	NS
Aspirin	12.0%	
Levodopa	16.0%	<.05
Aspirin and Levodopa	5.6%	
Aspirin	12.0%	<.05
Aspirin and Levodopa	5.6%	

*Chi-square test

In conclusion, two-hundred and two adult, female Sprague-Dawley rats (215 ± 11 g) were compressed to 100 psi (95% N_2 and 5% O_2) for 30 min and decompressed in 3 min. Forty percent of the control animals developed severe clinical signs of decompression sickness (DS) while 31% of the control animals died. Pretreatment with levodopa (4 days at 10 mg/kg i.p.) or aspirin (30 days at 40 mg in drinking water) decreased the occurrence of DS to 28% or 22% respectively while mortality was decreased to 16% or 12%. When levodopa and aspirin were given as a combination pretreatment the incidence of DS was decreased to 11% and mortality to 5.6% only. The increased beneficial effect of combination pretreatment (levodopa and aspirin) suggests the two drugs operate via different protective mechanisms. (See Addendum VIII for Manuscript in pres describing this work).

Figure 2. Blood sampling from an unanesthetized chronically cannulated rat.

III. Published Work of the Principal Investigator for the 1976-81 Period

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In Press

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ADDENDUM V

EFFECT OF LEVODOPA AND OF DOPAMINE ON ARTERIAL BLOOD PRESSURE IN HYPOTHERMIC RATS

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Running Title: Catecholamines in Hypothermia

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Abstract

Pava Popovic, V. Popovic and C. Honeycutt. Effect of levodopa and of dopamine on arterial blood pressure in hypothermic rats.

Administration of a large dose of levodopa or of dopamine increases the arterial blood pressure of normothermic rats. In this work, the effect of levodopa, dopamine or of norepinephrine on mean arterial blood pressure was studied in hypothermic rats. Levodopa, dopamine or norepinephrine were administered to unanesthetized rats through chronically implanted aortic cannula inducing maximal pressor response. At a body temperature of 17°-18°C, norepinephrine induced a 15 min rise in arterial blood pressure (from 76 to 132 mm Hg). After levodopa administration, the pressor response (from 82 to 117 mm Hg) lasted much longer, a full four hours. The dopamine's pressor response (from 68 to 103 mm Hg) persisted four hours also, but with a 20 mm Hg dip at 45 min. In animals that were rewarmed from hypothermia, the arterial blood pressure after levodopa stayed at the elevated level until body temperature reached 21°C. At this body temperature, at which shivering begins, the blood pressure suddenly decreased to low (control) hypothermic level of 78 mm Hg. After dopamine administration there was no decrease in arterial blood pressure during rewarming. The mechanisms that are responsible for long lasting pressor responses in hypothermic rats as well as cessation of these responses during rewarming are still unknown. It is possible that inactivation of the enzymes induced by body cooling below 21°C is a major cause of the prolonged pressor responses induced after levodopa or dopamine administration.

Cardiovascular effects of levodopa and dopamine have been studied extensively in several animal species and in man. In clinical practice, levodopa is used mainly in the treatment of Parkinsonism (Whitsett and Goldberg, 1972; Elliott et al., 1974; Lesser et al., 1979; Rosin et al., 1979). Given orally to patients, levodopa sometimes induces orthostatic hypotension. Administration of levodopa or of dopamine induces mesenteric, renal and cerebral vasodilation (Robie et al., 1974; von Essen and Roos, 1974; Dressler et al., 1975; Goldberg and Toda, 1975; Volkman et al., 1977). When administered in large doses, levodopa or dopamine increases arterial blood pressure (Goldberg and Whitsett, 1971; Pendleton and Setler, 1977; Popovic et al., 1977). It has been demonstrated in rats that the effect of levodopa on arterial blood pressure can be modified by anesthesia (Bevan et al., 1973; Popovic et al., 1977). Beneficial effects of levodopa and dopamine have been demonstrated in cardiogenic (Goldberg et al., 1977; Stephens et al., 1978) and noncardiogenic shock (Samii et al., 1978). The major benefit obtained with dopamine treatment seems to be an improvement in the perfusion of vital organs.

Cardiovascular effects of levodopa are attributed to a neurotransmitter substance dopamine, a metabolite of levodopa and precursor of norepinephrine (Blaschko, 1959; Hornykiewicz, 1973). The peripheral actions of dopamine are similar to those of norepinephrine. Dopamine plays an important role in the regulation of arterial blood pressure and also in the regulation of heart activity (Goldberg and Whitsett, 1971; Goldberg, 1975; Holloway et al., 1975; Stephens et al., 1978; Shearer and Caldwell, 1979). Dopamine stimulates beta-adrenergic receptors of the heart but in the smooth muscle of the systemic vascular bed dopamine acts on alpha-adrenergic receptors or possibly on the specific dopamine receptors (Goldberg, 1972, 1975a, b and 1978; von Essen and Roos, 1974).

In this work, the effects of levodopa and of dopamine on mean arterial blood pressure were studied in hypothermic rats. It is known that hypothermia decreases biological processes and exerts protective effects in many physiologically detrimental situations (Popovic and Popovic, 1974). However, the effects of low body temperature on the cardiovascular receptors are not well understood (Ahlquist, 1977; Karow, 1977; Bergh et al., 1979; Marshall and Stoner, 1979). In other words, the question arises whether the receptors retain their characteristic responses at low body temperatures. Furthermore, the enzyme activity is often altered at low body temperatures. To study the effects of hypothermia on cardiovascular receptors, levodopa, dopamine or norepinephrine were administered to normothermic rats in doses that produce a significant pressor response. The results were compared with responses obtained in hypothermic animals that received the same drugs.

Methods

Eighty-eight adult female Sprague-Dawley rats (200 ± 10 g) were used in the experiments. The rats were individually housed in cages and had food and water ad libitum. The aorta of each animal was chronically cannulated (Fluothane* anesthesia) with a PE 10 catheter via the left carotid artery at least two weeks prior to the blood pressure measurements (Popovic and Popovic, 1960; Popovic et al., 1963, 1977, 1978). During blood pressure measurements, the normothermic animal was placed in a transparent plastic box (4" x 10" x 4"). A longitudinal opening in the middle of the box cover permitted connection of the chronically implanted cannula (through a needle adapter) to a pressure transducer (P 23 De, Statham) and a polygraph (Beckman) as well as the free movement of the animal.

*Kindly supplied by the Ayerest Company.

The arterial blood pressure was recorded only during complete rest of the animal. The duration of the blood pressure measurement lasted from one to four hours depending on the experimental procedure. The arterial blood pressure measurements were done at the same time of the day. Hypothermia was induced by the hypoxic-hypercapnic cooling technique and the cooling was initiated with light anesthesia. After cooling, the animals were kept at a body temperature of 17°-18°C, by placing the animals on an ice tray. The body temperature was continuously monitored (Honeywell recorder). The animals were rewarmed to normothermia by an infrared lamp. Levodopa (Hoffman-LaRoche, 20 mg/kg), or dopamine (Sigma Chemical Company, 5 mg/kg) were dissolved in saline and administered in a single dose through the chronic aortic cannula with the volume not exceeding 0.5-0.6 ml. Norepinephrine (Levophed, Wintrop Laboratories, 0.1 mg/kg) was injected through the same chronic aortic cannula (0.03 ml in saline).

All animals were divided in eleven groups, each group having eight animals.

1. Norepinephrine administration to normothermic animals. The arterial blood pressure of normothermic animals was recorded before and after administration of norepinephrine.

2. Norepinephrine administration to hypothermic animals. Hypothermic animals (body temperature at 17°-18°C) received the same amount of norepinephrine as the normothermic rats. The hypothermic arterial blood pressure was recorded during four hours after administration of the drug.

3. Levodopa administration to normothermic animals. The arterial blood pressure of normothermic rats was recorded before and after levodopa administration.

4. Levodopa administration to hypothermic animals. The arterial blood pressure of hypothermic animals was recorded before levodopa administration. After levodopa injection the blood pressure was monitored for four hours.

5. Dopamine administration to normothermic animals. The experimental procedure was the same as in normothermic rats that received levodopa.

6. Dopamine administration to hypothermic animals. The same procedure was used as in the hypothermic group that received levodopa.

7. Hypothermic control animals. Animals in this group did not receive any drug. The arterial blood pressure was recorded in hypothermic rats for four hours.

8. Hypothermic animals during rewarming. The arterial blood pressure was recorded every five minutes during infrared rewarming of the animals until the body temperature returned to normothermic level.

9. Norepinephrine administration during rewarming from hypothermia. The arterial blood pressure was measured in hypothermic rats before and immediately after administration of norepinephrine. After the pressure response attained its peak, the rats were placed under an infrared lamp and warmed to a normal body temperature while their blood pressure was measured every five minutes.

10. Levodopa administration during rewarming from hypothermia. The arterial blood pressure was recorded in hypothermic animals before and after administration of levodopa. When the pressor response reached its peak, the animals were placed under an infrared lamp. The arterial blood pressure was then recorded every five minutes until the animal reached normal body temperature.

11. Dopamine administration during rewarming from hypothermia. Arterial blood pressure was recorded in hypothermic animals before and after administration of dopamine until the peak pressure response. Then the infrared rewarming was initiated while the arterial blood pressure was monitored until rewarming to normothermia.

Results

1. Norepinephrine administration to normothermic animals. The mean arterial blood pressure of the normothermic rats (117 ± 3 mm Hg) increased after norepinephrine administration to 163 ± 16 mm Hg. The peak pressure was reached 15-60 seconds after administration of norepinephrine. Four to five minutes later the blood pressure returned to the normal value (Fig. 1).

2. Norepinephrine administration to hypothermic animals. The animals were cooled to a colonic temperature of 17°-18°C. The arterial blood pressure was 76 ± 12 mm Hg. After administration of norepinephrine, the arterial blood pressure increased and reached the maximum response after five minutes (131 ± 13 mm Hg). The mean arterial blood pressure returned to the hypothermic level 15 minutes later (Fig. 1).

3. Levodopa administration to normothermic animals. After levodopa was administered, the peak pressor response (176 ± 3 mm Hg) was observed after 3-5 min (Fig. 2). Fifteen minutes later, the animals returned to the normal blood pressure. After returning to the normal level, the arterial blood pressure stayed continuously unchanged.

4. Levodopa administration to hypothermic animals. The blood pressure of hypothermic rats after levodopa administration was increased from 81 ± 3 mm Hg to 117 ± 7 mm Hg. The peak pressor effect was reached after 15 min. The blood

pressure stayed at the hypertensive level for four hours (Fig. 2). The experiment was terminated after four hours since longer lasting hypothermia brings cardiovascular deterioration and death to the animal (Popovic and Kent, 1965).

5. Dopamine administration to normothermic animals. After administration of dopamine to normothermic rats, the peak pressor response (163 ± 4 mm Hg) was reached in 30 sec. The pressor response returned to the normal level (114 ± 4 mm Hg) in five minutes and remained at that level for four hours (Fig. 3).

6. Dopamine administration to hypothermic animals. After administration of dopamine the arterial blood pressure of hypothermic animals increased from 67 ± 6 mm Hg to 103 ± 13 mm Hg. The peak pressor effect was reached after fifteen minutes. The blood pressure stayed elevated for four hours (Fig. 3) with an insignificant decrease observed at 30-60 minutes.

7. Hypothermic control animals. The mean arterial blood pressure was recorded every five minutes for four hours. The arterial blood pressure stayed as the low level 68 to 75 mm Hg (Figs. 2 and 3).

8. Hypothermic animals during rewarming. During infrared rewarming the arterial blood pressure increased from 72 ± 6 mm Hg to 108 ± 5 mm Hg (Figs. 5 and 6).

9. Norepinephrine administration during rewarming from hypothermia. Norepinephrine was administered to hypothermic rats at a body temperature of 17°-18°C. The maximal pressor response (115 ± 6 mm Hg) was observed after five minutes. At this time, infrared rewarming of the animal was initiated. Fifteen minutes later the arterial blood pressure returned to the control hypothermic value (Fig. 4). During rewarming to normothermia the arterial blood pressure in these animals was similar to the values observed in control hypothermic rats during rewarming.

10. Levodopa administration during rewarming from hypothermia. After levodopa was administered to hypothermic rats the maximal pressor response (111 ± 10 mm Hg) was reached after 30 min. During rewarming the arterial blood pressure stayed at the elevated level until a body temperature of 21°C. At this body temperature the mean arterial blood pressure decreased suddenly to the control hypothermic level of 79 ± 12 mm Hg. After this the arterial blood pressure followed the same curve as in hypothermic control animals (Fig. 5).

11. Dopamine administration during rewarming from hypothermia. When dopamine was administered instead of levodopa, the peak pressor response was reached after fifteen minutes, at which time rewarming of the animal was initiated. During rewarming, the arterial blood pressure stayed elevated until the animals became fully normothermic (Fig. 6).

Statistical analysis of the groups is shown in Table 1.

Discussion

Experimental work in animals and in man dealing with the cardiovascular effects of levodopa has shown that levodopa induces either hypotension or hypertension. Large doses of levodopa or of dopamine cause an increase in the arterial blood pressure. It has been suggested that the hypotensive effect of levodopa is centrally mediated (Henning and Rubenson, 1970; Schmitt et al., 1972). In unanesthetized rats, levodopa increases arterial blood pressure (Popovic et al., 1977). In this work, levodopa or dopamine administered to unanesthetized rats at a body temperature of 17°-18°C led to a prolonged hypertensive episode lasting four hours. When norepinephrine was injected, the pressor response was much shorter lasting, only fifteen minutes. Therefore, it seems that levodopa or dopamine have a different mechanism of action in

hypothermia than administered norepinephrine, a problem that merits further investigation. Thus, hypothermia seems to offer a new approach to the study of the effects of levodopa, of dopamine and of enzymes involved in their metabolism especially since the effects of these drugs on receptors can be investigated by use of specific enzyme inhibitors (Robson, 1971). It may be that in hypothermia levodopa is not metabolized to the level of norepinephrine. Furthermore, dopamine beta hydroxylase might be inactive at the low temperature or it might not be released. Finally, since dopamine has the same long lasting pressor effect as levodopa it may be that dopamine is not degraded completely, thus the effect of the MAO enzyme is potentiated.

During rewarming of rats, at body temperature of 21°-22°C at which the thermoregulatory mechanisms start to function again, the pressor effect of levodopa disappears suddenly. During further rewarming to normal body temperature, the arterial blood pressure follows the same curve as in control hypothermic animals. Thus, our results suggest that hypothermia potentiates the pressor action of levodopa or of dopamine by prolonging their effects. Levodopa's pressor effect is most likely mediated peripherally through direct dopamine action on vascular receptors. Dopamine does not cross the blood-brain barrier and the pressor effect of dopamine in hypothermic rats is of the same duration as the pressor effect of levodopa. The results are suggesting that enzyme decarboxylase is inactive at low body temperature. At body temperatures 21°-22°C, with the onset of shivering, the pressor effects of levodopa are abolished.

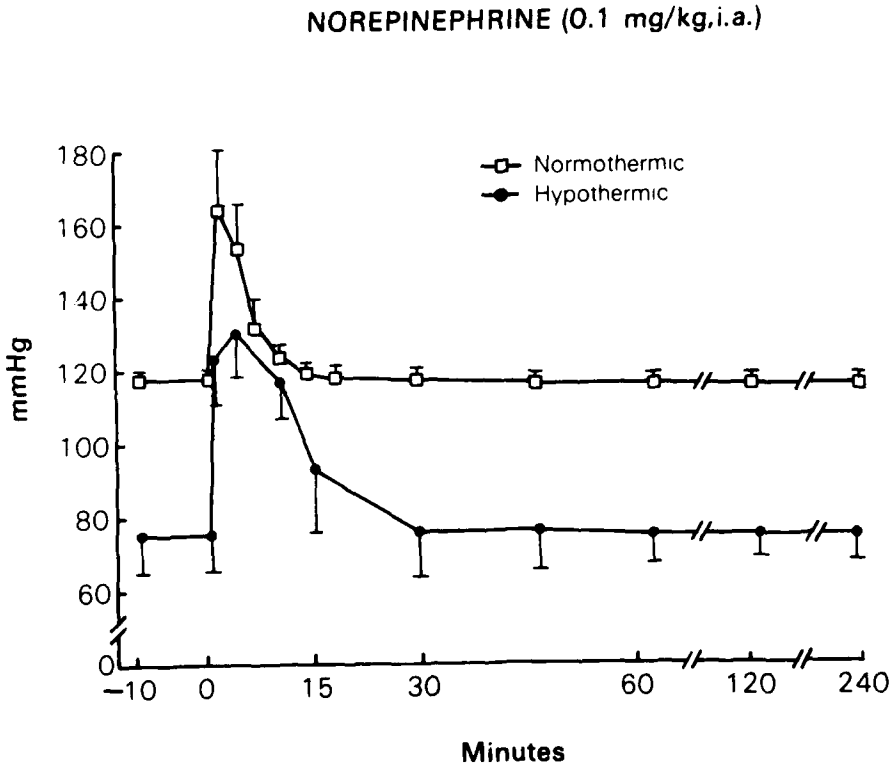

Fig. 1. Arterial blood pressure after administration of norepinephrine (0.1 mg/kg, i.a.) to normothermic and to hypothermic rats.

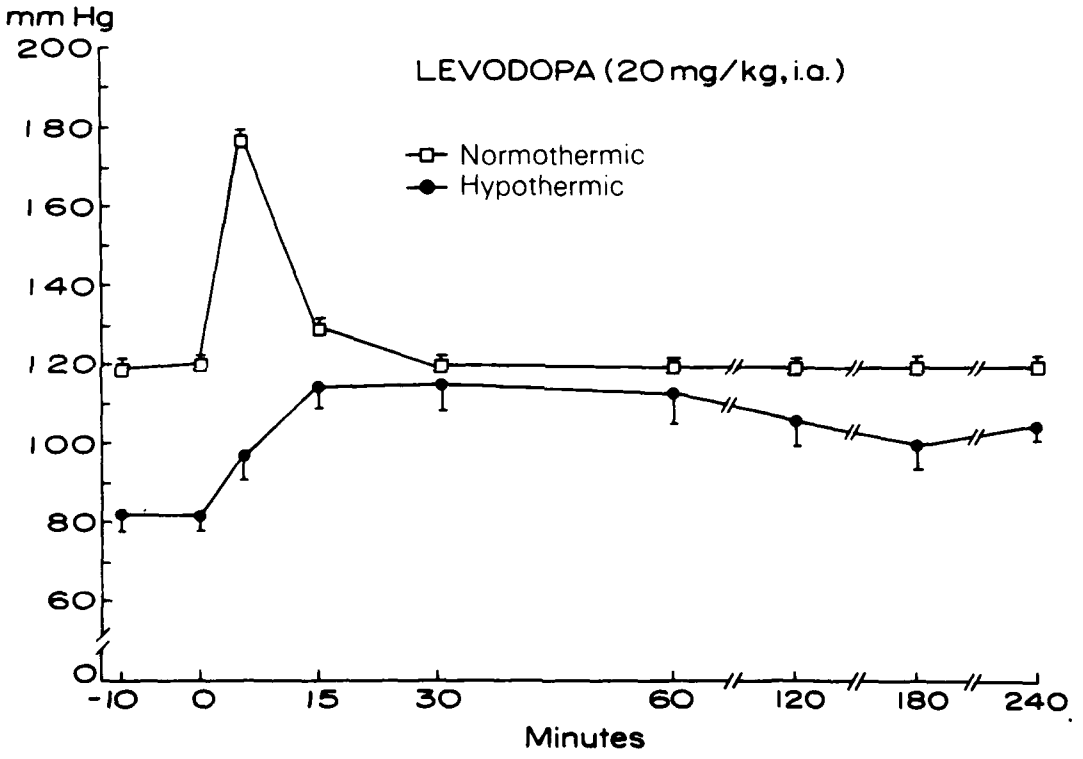

Fig. 2. Pressor response after levodopa administration (20 mg/kg, i.a.) to normothermic and to hypothermic rats.

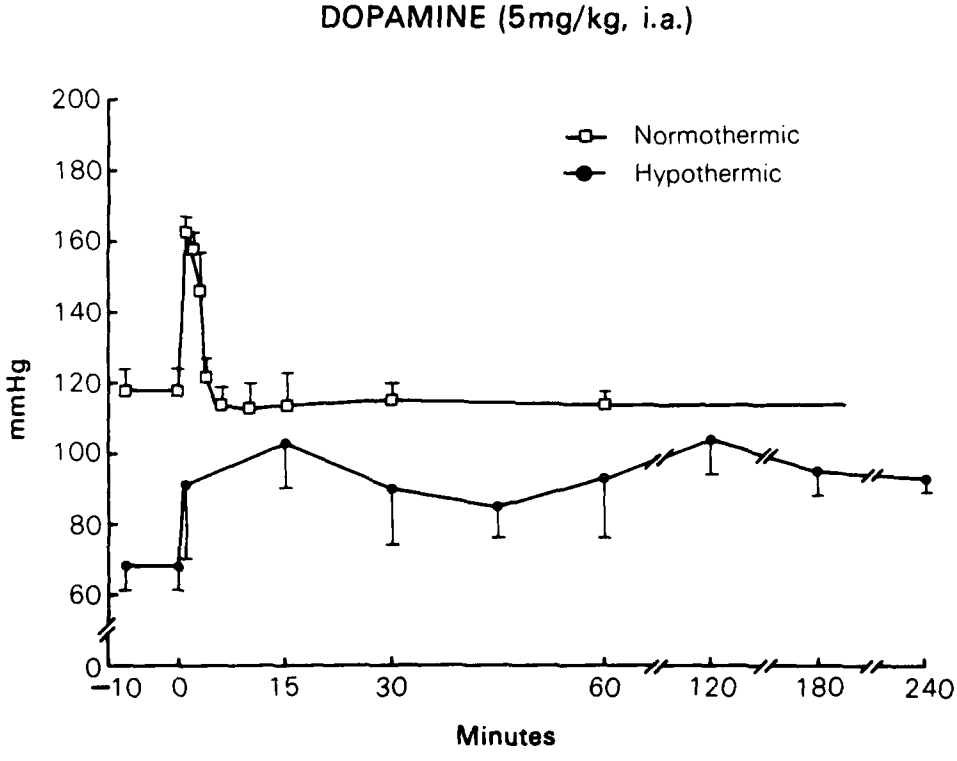

Fig. 3. Arterial blood pressure after dopamine administration (5 mg/kg, i.a.) to normothermic and to hypothermic animals.

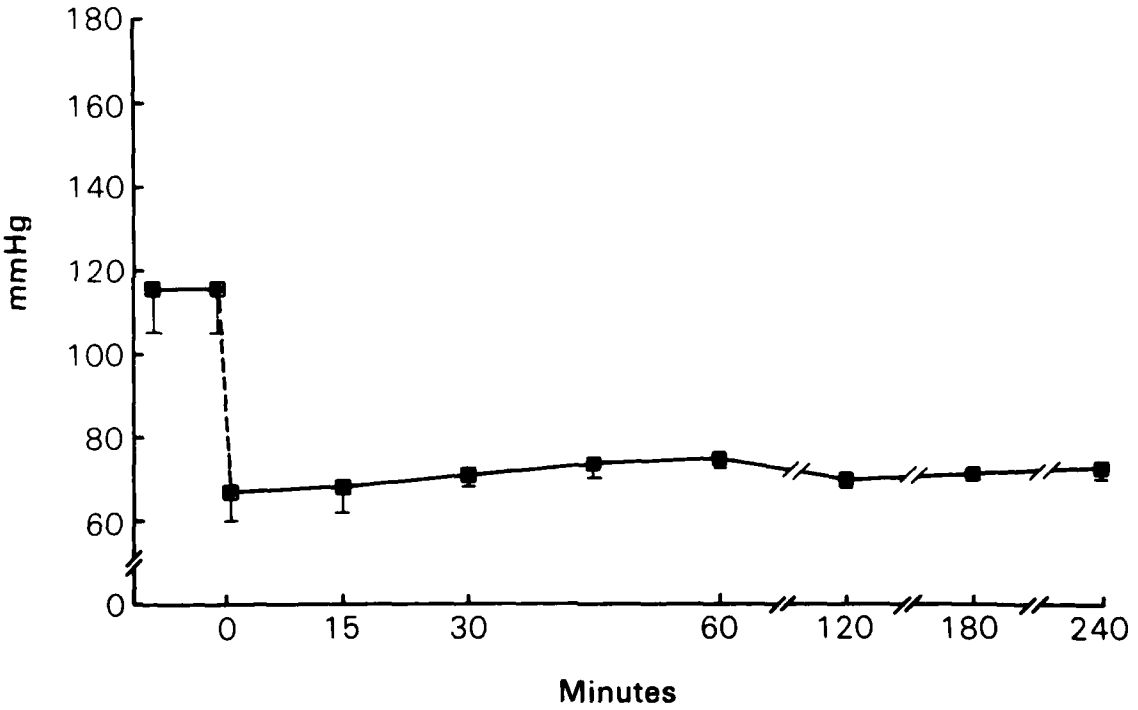

Fig. 4. Arterial blood pressure in the rats at a body temperature of 17°to 18°C.

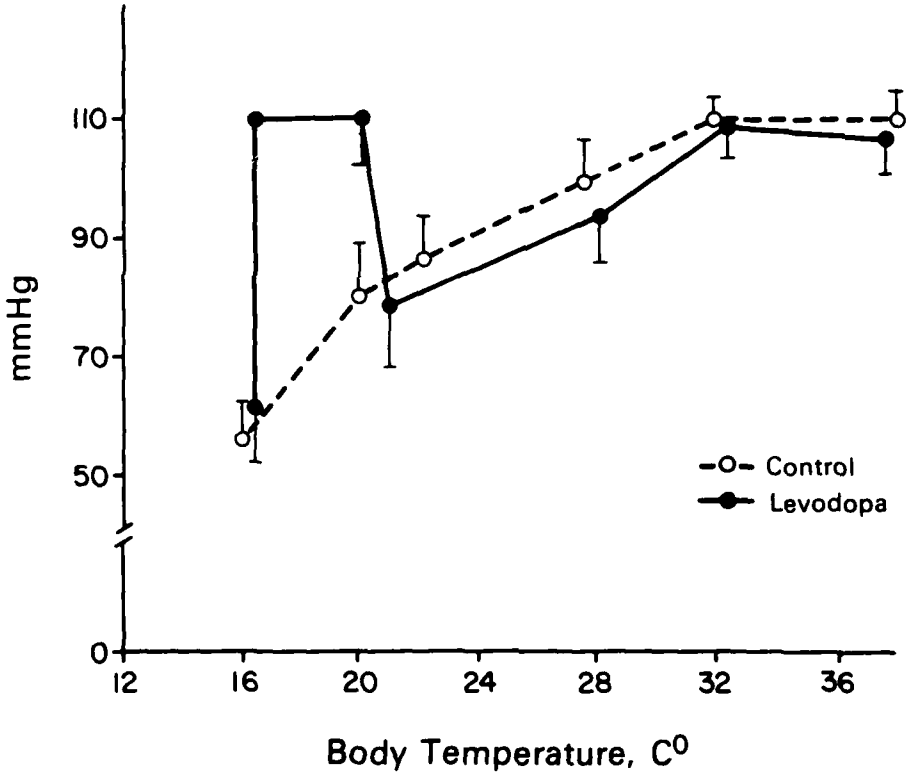

Fig. 5. Rewarming from hypothermia after levodopa administration.

GROUPS	TEMPERATURE			
	17°C	20°C	24°C	28°C
Control vs. Levodopa	- (12)*	++ (12)*	- (12)*	- (12)*
Control vs. Dopamine	++ (13)*	++ (13)*	++ (13)*	++ (13)*
Control vs. Norepinephrine	++ (10)*	- (9)*	- (9)*	- (10)*
Levodopa vs. Dopamine	++ (13)*	- (13)*	++ (13)*	+ (13)*
Levodopa vs. Norepinephrine	+ (10)*	+ (9)*	- (9)*	- (10)*
Dopamine vs. Norepinephrine	+ (11)*	++ (10)*	- (10)*	- (11)*

Table 1. Student t test for non-paired data

* Degrees of freedom

+ = significant ($P \leq .05$)
++ = highly significant ($P \leq .01$)
- = non-significant ($P \geq .05$)

A D D E N D U M V I

ADDENDUM VI

CIRCADIAN RHYTHM AND 5-FLUOROURACIL TOXICITY IN C_3H MICE

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Running Head: Circadian rhythm and toxicity of 5 FU

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INTRODUCTION

In man and in other diurnal homeotherms, body temperature, metabolism, secretory rates of hormones (for instance glucocorticoid secretion) and many other physiological processes are higher in the morning or in the afternoon hours than during the night. When a person changes daily working and sleeping habits, the cycle changes correspondingly (1). Thus in order to quantitate physiological parameters, the measurements must be done always at the same time of the day. Otherwise even basal (resting) value of a measured parameter could be two-three or more times higher at one time of the day than at the other. The 24-hour cyclic alteration of physiological processes in our body is induced by so called circadian rhythm. One might conclude, therefore, that because of the circadian variations the effectiveness of an administered drug is higher at some hours of the day and lower at others. The effect of circadian rhythms on toxicity of anticancer drugs have not been studied yet although one might expect that the rhythm alters the toxicity of these agents as well. In order to evaluate this problem the effect of daily rhythm on toxicity of an anticancer drug, 5-fluorouracil (5 FU) was administered to adult female C_3H mice, at different times of the day and night. Mortality of the mice was used as a criterion of the 5 FU toxicity during a period of 21 days after administeration of the drug.

MATERIALS AND METHODS

Seventy seven adult C_3H female mice (24.3±1.3g) were used in these experiments. To establish the toxicity levels, 5 FU was administered intraperitoneally (i.p.) to the animals in the amount of 100, 200 and 250 mg/kg. This was done at noon of the same day to three animal groups. Each group consisted of seven animals. While 100 mg/kg of 5 FU had a small toxic effect, 250 mg/kg was highly toxic. The dose 200 mg/kg of 5 FU was chosen as the most suitable for our experiment.

After body weight measurements, all animals were randomly divided into 8 groups, each containing seven mice. A single dose of 5 FU (200 mg/kg) was then administered i.p. to the first group at noon (12 a.m.). This was followed by administration of the same dose of 5 FU to other groups of animals at 4 p.m., 8 p.m., 12 p.m., 4 a.m., and 8 a.m., 12 a.m., and 4 p.m. of the next day. Body weights and animal mortality were observed daily during the following 21 days.

RESULTS

Administration of 5 FU at noon of the first day led to mortality of only two of seven mice (Table 1). The five remaining animals regained body weight and survived the 21 day long experiment. Higher mortality was observed when 5 FU administered at 4 p.m., 8 p.m., midnight and 8 a.m. The most toxic effect of 5 FU, with 100% mortality, was observed when the drug was administered at 4 a.m. Mortality of mice at 12 a.m. and 4 p.m. on the second day of the experiment was the same as in the first two groups of the animals.

DISCUSSION

Time is a dimension of life and rhythms are fundamental property of living matter, as Halberg (2) said. When the period of rhythmic alterations is approximately of the duration of an earth's rotation, it is called circadian rhythm. The clocks regulating these rhythms are endogenous and very precise. Existence of circadian variability in resistance and susceptibility to endotoxin and to drugs as well as in rejection of kidney allografts (3) has been already shown. In this work we demonstrated that toxicity of an administered anticancer drug, 5 FU, is different at various times of the day. The mortality of adult C_3H mice was 30 percent during inactive (12 a.m.) hours of the day when body temperature, metabolism, secretion and excretion are at the lowest point. The mortality was higher at late afternoon and during night hours reaching the peak, 100 percent, at 4 a.m. when most measured physiological parameters reach the highest point in this nocturnal animal.

Though the nature of the circadian rhythm and most of the underlying mechanisms of the rhythm are not yet known, on the basis of this work it is clear that the effectiveness of the anticancer drugs (measured here as mortality after administration of the 5 FU) depends largely on the hour of the day when the drug was administered. Our results suggest that in a man the same dose of an administered anticancer drug would be expected to be more effective in the late afternoon hours than in the early morning hours of the day. Furthermore, this work illustrates the importance of adhering strictly to predetermined biological rhythm when administrating a drug, especially when using a very toxic substance such as an anticancer agent.

SUMMARY

In order to study effect of daily (circadian) rhythm on toxicity of an anticancer drug, 5-Fluorouracil, adult female C_3H mice were used in this work. A dose of 200 mg/kg of 5 FU was administered intraperitoneally to the animals. Body weights and animal mortality were observed daily during the following 21 days after administration of the drug.

The most severe toxic effect of 5 FU, with 100% mortality, was observed when the drug was administered at 4 a.m. while at 12 a.m. 5 FU was least toxic. Since C_3H mice are nocturnal animals, they are at the peak activity in the early morning hours of the day. Thus, it seems that an increased 5 FU toxicity in C_3H mice coincides with an increased metabolic rate and other physiological processes of these nocturnal animals.

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Time	12 a.m.	4 p.m.	8 p.m.	12 p.m.	4 a.m.	8 a.m.	12 a.m.	4 p.m.
7 Day Mortality	2/7	5/7	5/7	2/7	7/7	4/7	1/7	5/7
21 Day Mortality	2/7	5/7	5/7	4/7	7/7	4/7	2/7	5/7

Table 1. Mortality (numerator) of C_3H mice receiving 200 mg/kg of 5 FU i.p. at different times during a 24 hour period. Each group consisted of seven animals (denominator).

ADDENDUM VII

GELATIN PRETREATMENT IN EXPERIMENTAL DECOMPRESSION SICKNESS

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POPOVIC, PAVA and V. P. POPOVIC. Gelatin pretreatment in experimental decompression sickness.

One hundred and eight Sprague-Dawley female adult rats were compressed to 100 psi (5% O_2, 95% N_2) for 30 min and decompressed within 2 min. After a 30 min dive in a group of 36 control animals, 19 developed decompression sickness (8 rats died). The experimental animals were pretreated with a single intra-arterial injection of 3% gelatin (25 ml/kg body weight). In this group of 36 rats, only 3 showed clinical symptoms of DS (1 rat died). The difference between the control and the gelatin pretreated groups was highly significant ($P < .01$). Saline pretreated animals faired somewhat better than the animals in the control group, but the difference between the two groups was not statistically significant. The beneficial effect of gelatin may be attributed to the coating of circulating platelets and of small blood vessels or to the coating of air bubbles that develop during rapid decompression.

Rat; chronic aortic cannula; 30 min compression.

INTRODUCTION

Processes and mechanisms that lead to decompression sickness (DS) are not well understood. Until recently it was thought that the mechanical action of bubbles was the main cause of DS. New data indicate that the process is more complex and that many pathophysiological changes occur during rapid decompression.

Surface activity at the blood-gas interface (Elliott et al., 1974) and intravascular bubble-platelet interaction (Philp, 1974) have been proposed as principal factors in the development of DS. The relationship between the amount of bubbles in the blood and the severity of DS is still not well understood. Thus, for instance, ultrasonic devices sometimes detect intravascular bubbles in divers with no apparent clinical manifestation of DS (Evans et al., 1972). The thrombocytopenia that follows platelet involvement was observed as early as one hour (Broussolle et al., 1973; Giry et al., 1977) but also a full twenty-four hours after the dive (Martin et al., 1973).

Presently, rapid recompression is the primary therapy for DS. Other treatments are induction of hypervolemia (Lamy and Hanquet, 1973), use of hypothermia (Thienprasit et al., 1975), and use of drugs. Drug therapy after DS has not been successful, though some improvement has been described after heparin administration (Reeves and Workman, 1971; Bennet, 1972; Cockett et al., 1972; Saumerez et al., 1973). It has been reported that the pretreatment with cyclohexanone HCP decreases incidence of DS in mice (Chryssanthou et al., 1971).

In the present work we wanted to study the effect of gelatin pretreatment on the development of clinical symptoms after rapid compression-decompression. The reason for choosing gelatin was that granulocytes,

extremely fragile white blood cells that are permanently damaged during freezing, survive freezing-thawing and actively phagocytize when protected with gelatin (Popovic et al., 1977 a,b). Thus, the idea arose that gelatin might also protect platelets from injury incurred in DS. Intravascular activation and increased utilization of platelets are probably one of the major causes of DS. In order to evaluate hypervolemia per se as an ameliorating factor in the onset of DS, another group of rats received the same volume of saline as the experimental animals.

METHODS

One hundred and eight female Sprague Dawley rats, weighing 185±10 g (S.D.), were used in the experiments. The aortic arch of all rats was chronically cannulated with PE 10 catheter two weeks prior to the experiment (Popovic and Popovic, 1960; Popovic et al., 1963).* The chronically implanted aortic cannulas remain patent for months (Popovic and Kent, 1964). In order to avoid circadian variations, all experiments (dives) were performed between 9 and 11 AM. The blood was sampled from the animals' chronic aortic cannulas one day before the experiment in order to eliminate the animals with a total WBC above or below the range (5,000-18,000/mm^3) and with more than 6,000/mm^3 granulocytes.

Before exposure to the compression-decompression profile, the animals were randomly divided into three groups according to close body weight match. The pretreatment (saline only or gelatin and saline) was administered through the cannula (4 min). Twelve animals (four controls, four saline pretreated,

*The animals with chronic aortic cannulas had after a 30 min dive (in over 200 experiments) the same clinical manifestations as noncannulated animals of the same age, weight, and sex.

and four gelatin pretreated rats), each in a separate plexiglass cage, were compressed in an experiment. The animals experienced a rapid dive in a 0.5 m^3 Vacudyne animal hyperbaric chamber (to 30 psi of air in 5 min, and then to 100 psi of 95% nitrogen, 5% oxygen in additional 7 min). The compression lasted 30 min while decompression lasted 2 min. After decompression, the animals in the same plexiglass cages were observed for the development of symptoms of DS.

Control animals. Thirty-six unrestrained, unanesthetized animals were exposed to the dive without pretreatment.

Saline pretreated animals. Prior to dive thirty-six animals were given saline (30 vol % of calculated blood volume, i.e., 25 ml/kg body weight) through the chronically implanted aortic cannula. This is the same amount of fluid that the rats in the experimental (gelatin pretreated) group received. The fluid was administered during a period of 4 min.

Gelatin pretreated animals. Thirty-six animals were exposed to the compression-decompression profile after administration of the gelatin solution (30 vol % of calculated blood volume of 3% gelatin in saline, i.e., 25 ml/kg body weight) through the chronic aortic cannula. The fluid was administered during a period of 4 min.

For statistical evaluations, contingency tables were prepared and Chi-square and P values were calculated in order to test the association between treatment and decompression sickness.

RESULTS

The results obtained after the 30 min dive are shown in Table 1. In the control group from thirty-six animals eight died, while in the experimental group from thirty-six gelatin pretreated animals only one succumbed to DS.

When animals with other DS symptoms (labored breathing and paraplegia) are included in evaluating the results, seventeen control rats in a group of thirty-six showed striking pathophysiological changes after compression-decompression while only three gelatin pretreated animals in a group of thirty-six developed similar symptoms. Administration of saline only (induction of hypervolemia per se) had some beneficial effect, but to a much smaller degree than a combination of gelatin and hypervolemia (Table 1).

Chi-square and P values obtained from the contingency tables indicate (a) no significant difference between untreated control rats versus saline pretreated animals with a 30 min dive, (b) a highly significant difference ($P < .01$) between untreated control rats versus gelatin pretreated animals, and (c) a significant difference ($P < .1$) between saline pretreated rats versus gelatin pretreated animals.

DISCUSSION

Hemostatic and hemodynamic changes that occur during rapid decompression have in recent years attracted the attention of an increasing number of investigators working in the field of DS. Philp and collaborators (1974) reported that thrombocytopenia observed after sudden decompression might be the consequence of an increased platelet consumption or of intravascular activation and increased utilization of platelets. It is also possible that platelet aggregation and sequestration lead to thrombocytopenia (Philp et al., 1974; Gray et al., 1975). Giry et al. (1977) observed a significant change in platelet turnover immediately after a dive: an increased production and release of platelets from bone marrow and from spleen with greater utilization of "new" (recently released) platelets which seem to be metabolically

more active than the old cells (Karpatkin and Sharmatz, 1969). The complex changes in the kinetics of platelets observed in the experiments of Giry et al. (1977) lead to a decreased platelet count in male rats one day after dive. A substantial reduction in platelet count and hypercoagulability in divers was also reported, while a decreased clotting time was described in animals after dive (Jacet et al., 1974).

These findings aroused new interest in the investigation of the platelet role in DS. Questions such as: What is the main site of removal of platelets from circulation or what is the specific role the platelets play in DS? are to be studied. Of course, the possibility that any tissue can be the main site of an increased utilization of platelets might explain the diversity of clinical pictures obtained after a simultaneous dive of a number of animals with similar physiological characteristics (sex, age, body weight, genetic properties). In such a case, while some animals do not show any clinical changes, others have an increasing number of symptoms of DS and some even die. The explanation for the animals' death would be that vital organs were involved to a greater degree than in other animals with smaller DS symptoms.

Our results show the beneficial effect of gelatin pretreatment in development of clinical manifestation of DS in rats. It seems likely that the beneficial effect of gelatin might be associated with a decreased injury to platelets. Coating properties, formation of artificial membranes over damaged cell membranes and embibition (trapping of water) were already suggested to explain the protective effects of gelatin in other situations. It is possible that in our experiment gelatin protects the platelets by coating which leads to a less striking intravascular platelet-gas bubble interaction. Similar beneficial effect of gelatin was already proven for granulocytes. These

white blood cells do not survive freezing-thawing injury. However, after gelatin pretreatment they are functionally viable after thawing and phagocytize yeast particles (Popovic et al., 1977 a,b). It is also possible that gelatin prevents the water escape from vasculature. Thus it might be beneficial as a result of plasma expanding properties. This is less likely, however, because other plasma expanders (for instance dextran), though somewhat beneficial, are much less effective than the gelatin reported here. In this work it has been also shown that hemodilution and hypervolemia lead to a somewhat improved clinical picture after a dive, but the difference between the two groups of rats (no pretreatment and saline pretreatment) was not statistically significant. Though after our results the mechanisms of gelatin's protective effects remain still unknown, it is clear that pretreatment with gelatin decreases clinical symptoms of DS after sudden decompression.

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Group	No. rats	Body weight, g	Animals with DS Symptoms			Animals with DS, %
			Labored breathing	Paraplegia	Total paralysis followed by death	
Control	36	186 ± 8	6	3	8	48
Saline pretreatment	36	184 ± 11	6	0	5	28
Gelatin pretreatment	36	185 ± 9	2	0	1	8

Table 1. Clinical manifestation of decompression sickness after a 30 min dive to 100 psi in control rats, in saline pretreated rats and in gelatin pretreated rats. All animals were grouped according to the most severe DS symptom.

ADDENDUM VIII

LEVODOPA AND ASPIRIN PRETREATMENT BENEFICIAL IN EXPERIMENTAL DECOMPRESSION SICKNESS

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The pathophysiology of decompression sickness (DS) is not well understood, though clinical signs of this condition were described by Robert Boyle three centuries ago (1). Today, it is speculated that the primary cause of DS is formation of gas bubbles in blood and other tissues (2,3,4). The clinical manifestations of fast decompression are unpredictable. Hematologic and humoral alterations are observed already in the early phases of blood-bubble interaction (5). The early symptoms lead usually to more severe clinical manifestations of DS. Widespread ischemia occurs, platelet survival is altered (6,7,8,9), neurological changes are observed (10) and in the most severe cases damage to the spinal cord or to the brain develops.

Rational therapy for DS has not been established. Pharmacological agents that might help prevention or treatment of DS are still sought (11). Discovery of a therapeutic agent beneficial in treatment or in prevention of DS would be of great practical importance since rapid recompression is the only successful therapy. However, compression chambers are not always available and any appreciable delay in the treatment of DS leads to failure. Therefore, it is speculated that pretreatment with choice agent prior to platelet changes and irreversible damage might be beneficial.

Sprague-Dawley female rats were used in the work. DS was introduced after 30 min compression and rapid decompression, with a mortality rate of 31% in the control animals. The experimental animals were pretreated with levodopa, a catecholamine that seems to be beneficial in recovery from paralysis induced by air embolism (12) and in decreasing coagulation (13), or with aspirin that effects platelet functions.

Materials and Methods. Animals. Two-hundred and two adult, female Sprague-Dawley rats weighing 215 ± 11 g S.D. were used in the experiments. All animals were housed individually and were given food and water *ad libitum*. Eighteen hours before decompression, the food was removed. The fasted, unanesthetized animals were weighted and placed in individual, perforated plexiglass cages (5" x 7" x 5") with ten cages contained in a plexiglass cage unit. The unit containing rats was placed in a noiseless Vacudyne animal hyperbaric chamber ($0.5m^3$) and the chamber prepared for a decompression (diving) regime.

Pressurization. The Vacudyne animal hyperbaric chamber with the animals was pressurized to 30 psi with compressed air at a rate of 6 psi/min. At the chamber pressure of 30 psi the compressed gas mixture was changed to 95% nitrogen/5% oxygen until 100 psi (at a rate of 10 psi/min). Thus the elapsed time of pressurization from 0-30 psi was 5 min and for pressurization from 30 to 100 psi an additional 7 min. Upon reaching a chamber pressure of 100 psi, a ventilation rate of 10 lit/min (with a driving pressure of 120 psi) was established and maintained throughout the duration of the compression (30 min). Chamber pressure was constant throughout the compression. Opening of a valve induced decompression at a rate of 10 psi/15 sec from 100 psi to 30 psi (2 min, 45 sec). At 30 psi a ball valve was opened permitting a rapid decompression from 30 psi to 0 psi (15 sec).

All dives were performed between one and four p.m. to avoid the effects of circadian variations. Ten animals were randomly chosen by close body weight match for exposure to a compression-decompression profile. The animals in compression chamber were observed through a window. After decompression the housing unit was taken out from the chamber and the undisturbed animals were surveyed for the development of DS symptoms.

Control animals. Forty-eight animals were exposed to the dive without any pretreatment.

Levodopa pretreated animals. Levodopa (10 mg/kg) was dissolved in warm saline and injected i.p. Fifty animals received the drug (at the same time of the day) for four days prior to compression-decompression experiment. The last administration of levodopa was just prior to the dive.

Aspirin pretreated animals. Aspirin (55 mg/kg) was dissolved in animals' drinking water. Fifty animals were kept on this regime for 30 days prior to the dive.

Pretreatment with levodopa and aspirin. Fifty-four animals in this group received a combination of levodopa and aspirin as pretreatment. The dose for levodopa was chosen because 10 mg/kg of levodopa induces pressor response (14).

For statistical evaluations, contingency tables were prepared and Chi-square and P values were calculated in order to test the association between treatment and decompression sickness. Clinical manifestations of DS in control groups of animals were highly reproducible.

Results. Control animals. Forty percent of the animals developed DS (Table 1). The mortality rate in this group was 31% (Table 2). Death occured at 6.3 ± 2.3 min after the decompression.

Levodopa pretreated animals. Twenty-eight percent of the animals in this group developed DS (Table 1), while the mortality rate was 16% (Table 2). Death of the animals was delayed to 21.2 ± 14.2 min after the decompression. There was a significant difference in mortality rate between this and the control group.

Aspirin pretreated animals. Twenty-two percent of the rats in this group developed DS after the dive (Table 1). The mortality rate was 12%. Death occured 9.5 ± 4.6 min after the decompression. A significant difference in mortality rate existed between this and the control group.

Pretreatment with levodopa and aspirin. After combination pretreatment eleven percent of the animals developed DS (Table 1). The mortality rate in this group was only 5.6% (Table 2). There was a significant difference in mortality rate between this and the control group. The onset of death was delayed to 15.2 ± 6.7 min.

Chi-square and P values obtained from the contingency tables indicate a) a highly significant difference ($P < .01$) of mortality rates between untreated control rats versus levodopa pretreated animals, b) a highly significant difference ($P < .01$) between untreated control rats versus aspirin pretreated animals, c) a highly significant difference ($P < .01$) between untreated control rats versus aspirin and levodopa pretreated rats, d) no significant difference between levodopa pretreated animals versus aspirin pretreated rats, e) significant difference ($P < .05$) between levodopa pretreated animals versus animals pretreated with aspirin and levodopa, and f) significant difference ($P < .05$) between aspirin pretreated rats versus animals pretreated with aspirin and levodopa.

Discussion. Search for a drug that might be used in the effective treatment of DS has not been successful. Furthermore, because DS develops abruptly, such a drug would be of little value if vital organs are damaged. However, a drug that fails in treatment of accute DS may still be successful if used as pretreatment. For this reason our interest was focused on agents

that might be effective in pretreatment, decreasing occurrence, severity and mortality of experimentally induced DS.

The blood-bubble interface in DS triggers platelet aggregation and damage to the vascular walls (15,16,17). The platelets seem to play a key role in the production of endothelial surface lesions (18). Furthermore, dead platelets increase hazards of coagulation in already hemoconcentrated blood of DS victims (19). In this work rats were pretreated with levodopa and with aspirin. Both drugs are clinically used and their effects well recognized. Aspirin was chosen as an inhibitor of platelet functions and platelet aggregation (20). Philp and coworkers have shown fewer changes in blood parameters after pretreatment with aspirin. However, aspirin had no effect on occurrence of DS. Levodopa was used because it is beneficial in Parkinsonism, in improving rigidity, in increasing locomotion (22), and as a potent vasoactive drug (23,24). Levodopa appears to be helpful in recovery from experimental spinal cord injury (25), and in recovery from air embolism induced paraplegia in rats (12). Our results show that aspirin or levodopa are beneficial when used as pretreatment in decreasing significantly clinical signs of serious forms of DS. When used in combination the effect of both drugs was even more striking. Instead of 31% mortality as in the control animals, the combination treatment decreased occurrence of death to a low value of 5.6%.

Summary. Two-hundred and two adult, female Sprague-Dawley rats (215 ± 11 g) were compressed to 100 psi (95% N_2 and 5% O_2) for 30 min and decompressed in 3 min. Forty percent of the control animals developed severe clinical signs of decompression sickness (DS) while 31% of the control animals died. Pretreatment with levodopa (4 days at 10 mg/kg i.p.) or aspirin (30 days at

55 mg/kg in drinking water) decreased the occurrence of DS to 28% or 22% respectively while mortality was decreased to 16% or 12%. When levodopa and aspirin were given as a combination pretreatment the incidence of DS was decreased to 11% and mortality to 5.6% only. Increased beneficial effect of combination pretreatment (levodopa and aspirin) suggests two different protective mechanisms of the used drugs.

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Table 1. Clinical manifestations of DS in rats after rapid decompression.

Group	No. rats	Body weight, g.	Animals with DS symptoms		Animals with DS, %
			Paraplegia	Total paralysis followed by death	
Control	48	202 16	4	15	40
Levodopa	50	212 16	6	8	28
Aspirin	50	214 14	5	6	22
Aspirin and Levodopa	54	212 15	3	3	11

Table 2. Composite statistical evaluation* of DS mortality in rats after pretreatment with levodopa and aspirin.

Animal Group	Mortality Rate	Level of Significance
Control Levodopa	31.3% 16.0%	<.01
Control Aspirin	31.3% 12.0%	<.01
Control Aspirin and Levodopa	31.3% 5.6%	<.01
Levodopa Aspirin	16.0% 12.0%	NS
Levodopa Aspirin and Levodopa	16.0% 5.6%	<.05
Aspirin Aspirin and Levodopa	12.0% 5.6%	<.05

*Chi-square test

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The main objective of this project is a study of the protective effects and mechanisms of levodopa and some other agents in the prevention and recovery of rats from decompression sickness. For better clarity, the description of performed work is divided into two parts-- air embolism studies and decompression sickness studies.

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ADDENDUM I

Levodopa-enhanced recovery from paralysis induced by air embolism

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In this study levodopa was chosen as an agent that might ameliorate the clinical manifestations of paralysis induced by air embolism because of reported effects of this drug on muscle spasticity and locomotion. In order to induce air embolism, the aorta of rats was cannulated chronically. Fifteen days later, after full recovery of the animals, air was administered through the chronic cannula into the descending aorta of each rat (0.35 ml. of air per 100 Gm. during 4 seconds). The paralysis of both hind legs was observed one to 10 minutes later. Only animals that had total paralysis of both hind legs, without any sensation, were used in the experiments. Levodopa was administered 2 minutes after experimentally induced paralysis. The levodopa treatment was repeated every day for 6 days. After 6 days, six levodopa-treated (intraperitoneally) animals in a group of eight and ten levodopa-treated (intra-arterially) animals in a group of 12 recovered fully from the paralysis. Only three animals in a group of 13 (untreated) or two animals in 12 (solvent administration) recovered without the levodopa treatment.

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AIR EMBOLISM that might occur during rapid decompression in divers or in pilots can lead to profound pathophysiological disturbances and sometimes can cause paralysis of the spinal cord. Exposure to compression chambers with consequent decrease in bubble size sometimes brings rapid relief of decompression sickness and appears to be helpful in other causes of air embolism. However, the compression chambers are few and eventual decompression is a very long process. It has been suggested that hypothermia might be useful in the case of air embolism in two ways: first, by decreasing the size of bubbles, and, second, by decreasing metabolism and danger from anoxia.[9, 14] However, cooling is a rather slow process. Administered heparin might be helpful in decompression sickness[3, 6, 11] because it prevents hemoconcentration.[10] Low-molecular weight dextran has a similar effect[4] by keeping circulating blood volume at a near normal level. However, neither of these two agents has been accepted in clinical practice.

After developing a suitable animal model system to study recovery from paralysis following air embolism, levodopa was chosen as the agent that might be helpful because of its well known effects on muscular spasticity and on locomotion.[15]

MATERIALS AND METHODS

Adult female Sprague-Dawley rats weighing 196 ± 16 grams (S.D.) were used in the experiments. Each animal was housed in a separate cage and was given food and water *ad libitum*. The aorta of each animal was cannulated under fluothane* anesthesia with a fine polyethelene (Pe 10) tubing via left carotid artery 2 to 3 weeks prior to the experiment.[12] After full recovery from operation and anesthesia, the animals continued to grow, following the standard curve of growth for this species.[13] In order to induce air embolism, air was injected through the aortic cannula into the descending aorta. With an airtight plastic tuberculin syringe, 0.35 ml. of air per 100 Gm. of body weight was injected during 4 seconds. The amount of air injected is a critical factor in inducing hind leg paralysis. More air usually causes a complete paralysis of the animal; less air leads only to a partial paralysis or has no effects. The right amount of administered air gave reproducible results in 70 percent of cases: total paralysis of both hind legs in a very short time (one to 10 minutes). Of the remaining 30 percent of the

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Fig. 1. Recovery (complete recovery of motor activity and of sensation) of rats after air embolism–induced paralysis of both hind legs.

animals, 15 percent had partial paralysis of hind legs and 15 percent had paralysis of the whole body. The hind leg paralysis is manifested by total muscular paralysis, loss of muscle tone, and total absence of sensation. During the injection of air emboli or during development of paralysis, the animals never evidenced distress.

In order to avoid any possible circadian effects, air embolism was induced between 9 and 11 A.M. only. Levodopa* was administered between 9 and 11 A.M. (either intraperitoneally or intra-arterially) and between 4 and 5 P.M. (intraperitoneally).

The judgment of recovery from hind leg paralysis was as follows: recovery of sensation and motor activity in an experimental animal was recorded as full recovery; partial recovery of one leg, either sensation or sensation and motor activity was recorded as one partial recovery.

The colonic temperature was monitored with thermocouples and a recorder.

After induction of total hind leg paralysis, 45 animals were separated randomly into four groups.

Untreated animals. Thirteen animals in this group were without treatment. Their body weight, colonic temperature, and eventual recovery from paralysis were monitored two times per day for 6 days.

Solvent administered animals. Twelve animals in this group were given daily solvent base (without levodopa) in the same amount as levodopa-treated (intra-arterially) animals (0.5N HCl buffered with 0.5N NaOH in saline, 1 ml. per kilogram of body weight, intra-arterially). Body weight, colonic temperature, and recovery from paralysis were monitored twice per day during a period of 6 days after induced paralysis.

Levodopa-treated (intraperitoneally) animals. Eight animals in this group received 10 mg. per kilogram of levodopa (in saline) intra-arterially 2 minutes after induced paralysis. The same day, in the afternoon, and during the additional 5 days, all animals received intraperitoneally 50 mg. per kilogram of levodopa dissolved in saline in the morning and afternoon until recovery. Body weight, colonic temperature, and recovery from paralysis were monitored twice per day during this period.

Levodopa-treated (intra-arterially) animals. Twelve animals in this group received 10 mg. per kilogram of levodopa (in solvent) intra-arterially 2 minutes after induced paralysis. The same day, in the afternoon, all animals received an additional intra-arterial administration (10 mg. per kilogram in solvent) which was continued daily for the next 5 days or until recovery. Body weights, temperatures, and recoveries were monitored as in the other groups.

RESULTS

After paralysis induced by air embolism, the body temperature of the animal always was decreased. Hypothermia already was apparent 5 to 15 minutes after air embolism and lasted until the recovery of the animals. The body temperature of paralyzed rats was between 29 and 34° C. Loss in body weight of all paralyzed animals was marked. Sometimes the loss was progressive, but in a few cases the paralyzed animals stabilized at a decreased body weight. After recovery

*Supplied by Hoffman-LaRoche, Inc., LaRoche Park, Nutley, N. J. 07119.

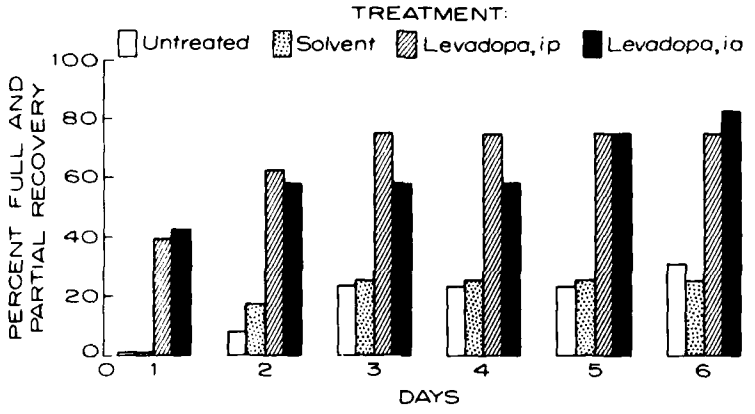

Fig. 2. Recovery (complete *and* partial recovery of motor activity and of sensation) of rats after air embolism–induced paralysis of both hind legs.

from paralysis, all animals regained the normal body weight in a few days.

Untreated animals. The spontaneous recovery of the animals in this group was slow. This applied to full recovery of both hind legs or to partial recovery of locomotion or of sensation in only one of the hind legs. Figs. 1 and 2 show the results.

Solvent-administered animals. The recovery of these animals after fluid-solvent administration was similar to that observed in the previous group. This applies to total recovery or to partial improvement from paralysis (Figs. 1 and 2).

Levodopa-treated (intraperitoneally) animals. The recovery occurred much faster and in more animals after intraperitoneal levodopa treatment than in two previous groups. (Figs. 1 and 2).

Levodopa-treated (intra-arterially) animals. The recovery from paralysis after intra-arterial administration of levodopa was similar to that in the intraperitoneal levodopa group (Figs. 1 and 2).

In order to test the null hypothesis that the variables (recoveries) being tested were independent of treatment, contingency tables for (1) individual treatment and (2) combined control vs. levodopa groups were prepared and analyzed according to chi square and probability statistics. In all instances the null hypothesis for both full and full and partial recovery was rejected at the $P < 0.05$ or $P < 0.01$ levels.

DISCUSSION

Air embolism can cause serious consequences. We chose to study the effect of levodopa on paralysis induced by air embolism because it is an injectable agent that decreases coagulation[1, 5] and because of its effect on muscle tone and locomotion.[15] Furthermore, there are reports that levodopa causes hypothermia[8] which has been proven beneficial in treatment of air embolism.[9, 14]

It is well known that neurocirculatory manifestations represent the most serious form of air embolism disorders.[7, 16] Furthermore, the systemic infarction of the spinal cord by air embolism is a condition from which recovery is slight and lengthy. For this reason we chose for our animal model system an air embolism–induced infarction of lower parts of the spinal cord as the most suitable for the study of recovery from experimentally induced paralysis of both hind legs.

The recovery after levodopa administration, either intraperitoneal or intra-arterial, was striking and significantly higher than in two control groups of animals (untreated or solvent administration). At present it is difficult to speculate upon the mechanisms of action of levodopa. Is levodopa a nonspecific agent and therefore effective for other kinds of spinal cord injury and clinical manifestations? Or is it possible that levodopa is effective in other types of spinal cord injury, not only those associated with anoxia? It might be that administered levodopa replaces norepinephrine at the site of spinal cord injury[2] and therefore prevents the development of hemorrhagic necrosis. Levodopa appears to prevent full development of anoxic damage. Whatever the case, the results obtained open, we believe, a new field in investigations of the pathology following air embolism and new hopes for increased recovery.

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Injectable Agent for the Treatment of Air Emboli-Induced Paraplegia in Rats

ADDENDUM II

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Department of Physiology, Emory University Medical School, Atlanta, Georgia 30322

POPOVIC, P., V. POPOVIC and R. SCHAFFER. *Injectable agent for the treatment of air emboli-induced paraplegia in rats.* Aviat. Space Environ. Med. 47(10):1073-1075, 1976.

Rats with air emboli-induced paraplegia were treated with daily levodopa injections (intra-arterially or intraperitoneally). Of the control animals, 20% fully recovered from paraplegia during a period of 7 d. Of the levodopa treated animals, 85% fully recovered during the same period. It appears, therefore, that levodopa might be one of the few injectable agents enhancing recovery from air-induced paraplegia, suggesting its possible use in decompression sickness.

MECHANICAL malfunctioning of the equipment during deep sea diving or during airplane flights might lead to decompression sickness in divers and pilots. During rapid decompression and consequent formation of air emboli (25), the hazard is great if air emboli reach vital organs. Derangements of the cardiovascular system and of the central nervous system might be very serious, sometimes irreparable. Use of compression chambers is an effective treatment against embolization, but compression chambers are not commonly available. If decompression sickness occurs while diving in remote places, or if decompression problems occur during airplane flights or during space flights (28,29), the most desirable treatment would be a fast acting, injectable drug therapy. Heparin and, in lesser degree, dextran were reported as somewhat beneficial in decompression sickness (3,7,26,30), but clinical results are not encouraging. Hypothermia might be beneficial also (32).

One of the most serious clinical forms of decompression sickness and air embolization is damage to the lower part of the spinal cord manifested as paraplegia. Treatment of paraplegia is a lengthy process, often without successful results. In order to study this problem, we developed an animal model of air emboli-induced paraplegia in rats. The idea was to use an injectable drug that might improve clinical symptoms or bring recovery from paraplegia. Levodopa was chosen because of its beneficial effects on muscle tone, motor function, and locomotion (2,14-17,24,27,33) and possibly on blood coagulation (1).

MATERIALS AND METHODS

Adult female Sprague-Dawley rats, weighing 196 ± 16 g (S.D.), were used in the experiments. Each animal was housed in a separate cage and was given food and water *ad libitum*. The aorta of each animal was cannulated under Fluothane anesthesia, kindly supplied by Ayerst Co., with a fine polyethelene (Pe 10) tubing via the left carotid artery 2-3 weeks prior to the experiment (21,22). After full recovery, the animals continued to grow, following the standard curve of growth for this species.

In order to induce paraplegia, air was injected through the aortic cannula into the descending aorta. With an airtight plastic tuberculin syringe, 0.35 ml of air per 100 g body weight was injected during 4 s. The amount of air injected is a critical factor in inducing paraplegia. More air usually causes a complete paralysis of the animal; less air leads only to a partial paralysis or has no effects. The right amount of administered air gave reproducible results in 70% of cases: total paralysis of both hind legs in a very short time (1-10 min). Of the remaining 30% of the animals, half had partial paralysis of the hind legs and half had paralysis of the whole body. The paraplegia is manifested by total muscular paralysis, loss of muscle tone and total absence of sensation. Air embolism was induced between 9 and 11 a.m. only. Levodopa, kindly supplied by Hoffman-LaRoche, was administered between 9 and 11 a.m. (either ip or ia) and between 4 and 5 p.m. (ip). The colonic temperature was monitored with thermocouples and a recorder.

Recovery of sensation and motor activity in both legs in an experimental animal was recorded as full recovery.

All animals that recovered from paraplegia survived.

After induction of paraplegia, 45 animals were separated randomly into two groups:

Control animals: 13 animals were without treatment after paraplegia. An additional 12 control animals with paraplegia were given daily solvent base (without levodopa) in the same amount and way (ia) as levodopa treated animals. Body weight, colonic temperature, and recovery from paraplegia were monitored twice per day during a period of 6 d.

Experimental animals: 12 paraplegic animals received 10 mg/kg levodopa in solvent (0.5 N HCl buffered with 0.5 N NaOH in saline) intra-arterially 2 min after established paraplegia. That afternoon, all animals also received an intra-arterial injection of levodopa (10 mg/kg in sovlent) which was continued daily for the next 5 d or until recovery. An additional eight experimental ani-

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mals received 10 mg/kg levodopa (in saline) intra-arterially 2 min after development of paraplegia. That afternoon, this group of animals received intraperitoneally 50 mg/kg of levodopa dissolved in saline. This treatment (ip) was continued every morning and afternoon for 6 d or until recovery. Body weight, colonic temperature, and recovery from paraplegia were monitored twice per day during this period.

RESULTS

After air embolism-induced paraplegia, the body temperature of all animals decreased to 29-32°C. This process occurred 5-15 min after embolization and lasted until the recovery of the animals. The body weight of all paraplegic animals markedly decreased. The loss was progressive except in a few cases when the body weight stabilized at a decreased level. After recovery from paraplegia, the animals regained normal body weight in a few days.

Control animals: The spontaneous recovery from paraplegia of the animals in this group was slow. This applied both to nontreated and solvent administered animals. Fig. 1 shows the results.

Experimental animals: The full recovery from paraplegia after intra-arterial administration of levodopa or after intraperitoneal administration of levodopa was faster and in more animals than in the control group. Results in Fig. 1 show that the full recovery from paraplegia after administration of levodopa (either intra-arterial or intraperiotoneal) was significantly higher than in control animals.

For statistical evaluations, Chi-square values were calculated from contingency tables prepared to test a) the homogeneity between control animals and the homogeneity between experimental animals, and b) the association between treatment and recovery. The observed heterogeneity Chi-square values indicated that the data were homogeneous and could be pooled. The Chi-square values obtained for each association test rejected the null hypothesis that recovery was independent of treatment at the $p<0.01$ level.

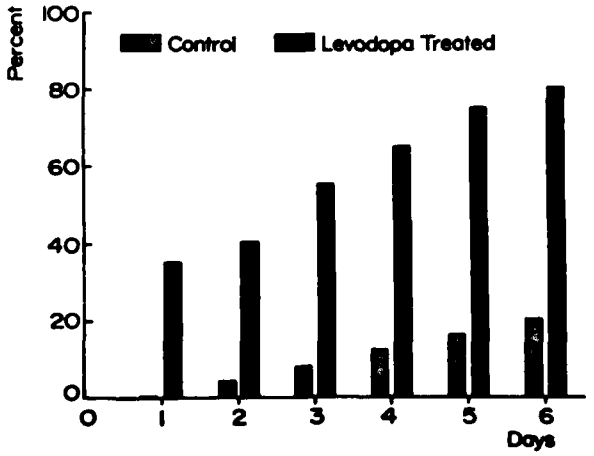

Fig. 1. Full recovery of motor activity and sensation of rats after air embolism-induced paraplegia.

DISCUSSION

Levodopa is used for treatment of spasticity and rigidity in Parkinson's disease (11). Recent reports describe the effect of this drug on motor function and locomotion in experimental animals (23). In our study, we postulated that levodopa might be beneficial for treatment of air emboli-induced paraplegia. It is well known that air emboli, which might occur during rapid decompression (10) can cause profound cardiovascular (5,6, 8,12,20,31) and profound central nervous system derangements. One of the most difficult consequences of rapid decompression and air embolization is paraplegia, paralysis of the lower part of the spinal cord. Recovery from paraplegia is a slow process, often without appreciable clinical success. Until now exposure to compression chambers was the only effective way for treatment. However, the chambers are not available in remote places or during space flights. Administered herparin and dextran (3,7,26,30) lead to some improvements in experimental decompression sickness but are not yet used successfully in clinical practice.

A fast, injectable drug therapy would be the most desirable for treatment of rapid decompression and its harmful consequences. It appears now that levodopa might be such an agent.

What is the action of levodopa in enhancing the recovery from air embolism-induced paraplegia? It is possible that after administration of levodopa, progressive hemorrhagic necrosis (4,9,18,19,34) at the involved part of the spinal cord does not develop fully and that anoxic damage to the affected parts of the spinal cord is diminished. Mechanisms of beneficial action of levodopa are not yet known.

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ADDENDUM III

Effect of Levodopa on Arterial Blood Pressure in Unanesthetized and in Anesthetized Rats (39678)

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The use of levodopa in the treatment of parkinsonism has led to an increased interest in the physiological and pharmacological effects of this drug, as well as to an awareness that cardiovascular and, to a smaller degree, other derangements occur during levodopa therapy. For this reason the effects of levodopa on the cardiovascular system have been studied extensively. The results obtained are somewhat contradictory. For example, it has been reported that levodopa decreases (1–3) or increases (4–9) the arterial blood pressure. The difference in results might be due to the choice of the animal species or to the difference in administered doses of levodopa (16, 32). Anesthesia and surgical procedures may also be factors in variability of the obtained results. It has been shown that anesthesia and surgery profoundly change cardiac output, arterial blood pressure, and heart rate in experimental animals (10).

This main purpose of this work was to study the acute effects of levodopa on the arterial blood pressure in unanesthetized and anesthetized rats after chronic aortic cannulation. The mean arterial blood pressure was recorded before and after single administration of levodopa.

Materials and methods. Forty-two adult female Sprague–Dawley rats weighing 198 ± 7 (SD) g were used in the experiments. Each animal was housed in a separate cage and was given food and water *ad lib*. The aorta of each animal was cannulated under halothane (Fluothane, kindly supplied by Ayerst Laboratories) anesthesia with a fine polyethylene tubing (PE 10) via the left carotid artery 3–4 wk prior to the actual blood pressure measurement (11, 12). After full recovery from surgery and anesthesia, the animals continued to grow following the standard curve of growth for this species (12). In addition to the chronic aortic cannula, six rats were also carriers of a chronic right ventricular heart cannula (PE 10) permitting the iv administration of levodopa (12).

During blood pressure recording, each animal was placed in a plastic 4″ × 10″ × 4″ box. A 1/4-in. wide opening in the middle of the cover of the box permitted connection of the implanted cannula to the transducer and free movement within the box during voluntary locomotion of the rat. The implanted cannula of the animal was connected by a needle adapter to a pressure transducer (P23 De, Statham) and polygraph (Beckman). The pressure was recorded after the animal reached a complete resting state, 5–15 min after placement in the box. Each measurement after levodopa administration lasted one hour.

Levodopa (Hoffman La Roche) was dissolved in warm saline and administered intra-arterially through the chronically implanted aortic cannula. In six rats the levodopa was administered iv through the right ventricular heart cannula. The volume of administered fluid (saline as a solvent) never exceeded 0.5 ml. The arterial blood pressure measurements were made between 9 and 12 AM. Besides the mean arterial blood pressure, body weight, body temperature, and hematocrit ratio were monitored. Nembutal (sodium pentobarbital) was given ip, 40 mg/kg. Halothane was mixed with oxygen and introduced through a mask placed over the nose and the mouth of the rat. The flow rate of halothane and oxygen was maintained at a constant level. Depth of anesthesia was judged by breathing rate and corneal blink reflex. However, variations in the respiration rate and the duration of anesthesia probably have effects on the depth of anesthesia. Levodopa was administered 10 min after the animals reached the desired levels of anesthesia.

Statistical treatment consisted of single factor analysis of variance on repeated

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measures. Significance at the $P = 0.05$ level was determined from the least significant difference procedure for pairs of means.

Animal groups. All of the rats were randomly separated into six groups, each group consisting of six animals. The groups were:

Unanesthetized rats. 1. 10 mg/kg levodopa, i.a. injection. 2. 20 mg/kg levodopa, i.a. injection. 3. 10 mg/kg levodopa, iv injection.

Anesthetized rats. 4. 10 mg/kg levodopa, i.a. injection and nembutal anesthesia. 5. 20 mg/kg levodopa, i.a. injection and nembutal anesthesia. 6. 10 mg/kg levodopa, i.a. injection and halothane anesthesia.

Results. Mean arterial blood pressure after a single intravascular injection of 10 mg/kg of levodopa. Administration of nembutal or of halothane decreased the arterial blood pressure from 120 mmHg to 80–85 mm Hg. After administration of levodopa (10 mg/kg, ia), the arterial blood pressure increased in unanesthetized and in anesthetized rats (Fig. 1). The rise in arterial blood pressure reached a peak after 2–5 min in all groups. The increase of the arterial blood pressure in unanesthetized rats 2–5 min after i.a. levodopa administration was between 35 and 50 mmHg (Fig. 2). Fifteen minutes after levodopa administration, the arterial blood pressure of the unanesthetized rats was close to the control preadministration level, where it stayed during the next 45 min. The return to control values was somewhat slower in anesthetized rats. Intravenous administration of levodopa caused a larger increase of the arterial blood pressure in unanesthetized rats than the intra-arterial injection of the same dose.

In the nembutal group the increase of the arterial blood pressure after 2–5 min was 90 mmHg, much greater ($P < 0.05$) than in other groups (Fig. 2 and Table 1). After levodopa administration, the increase of the mean arterial blood pressure was smaller with halothane than with nembutal anesthesia (Figs. 1 and 2).

The composite analysis of the observed changes of the arterial blood pressure induced by 10 or 20 mg/kg of levodopa is given in Table 1.

Mean arterial blood pressure after a single intra-arterial injection of levodopa (20 mg/kg). Levodopa (i.a.) caused hypertensive responses in unanesthetized and in anesthetized rats, with the peak response at 2–5 min postdrug administration (Fig. 3). The return to the control preadministration values was slower in anesthetized than in unanesthetized rats (Figs. 3 and 4). The increase of the arterial blood pressure was greater, 90 mmHg, in both nembutal anesthetized groups after levodopa (10 or 20 mg/kg, i.a.) administration than in unanesthetized animals (Fig. 4).

After intravascular administration of the same volume of saline (solvent for levodopa) to 12 animals, their arterial blood pressure stayed unchanged. Calculated on the 100% basis, before the saline administration, the values of the mean arterial blood pressure after saline injection were 100 ± 0.3% (SD).

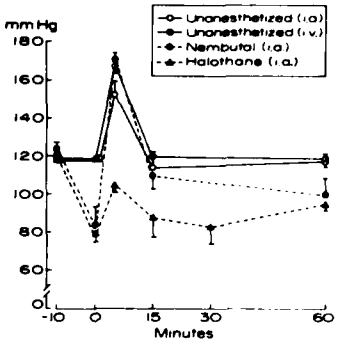

FIG. 1. Mean arterial blood pressure (±SE) of adult female rats after levodopa (10 mg/kg) administration (i.a., intra-arterial administration; iv, intravenous administration). Levodopa was administered at 0 min.

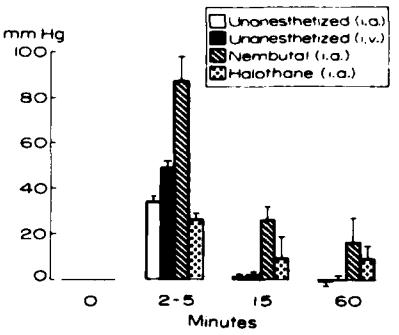

FIG. 2. Increases of the mean arterial blood pressure (±SE) of adult female rats after levodopa (10 mg/kg) administration (i.a., intra-arterial administration; *iv*, intravenous administration).

TABLE 1. COMPOSITE STATISTICAL EVALUATIONS* OF MEAN ARTERIAL BLOOD PRESSURE CHANGES 2-5 MIN AND 15 MIN AFTER LEVODOPA ADMINISTRATION TO UNANESTHETIZED AND ANESTHETIZED RATS.

Animal Group	Time, min					
Unanesthetized Levodopa, 10 mg/kg i.v.	2-5 15	NS NS				
Unanesthetized Levodopa, 20 mg/kg i.a.	2-5 15	<.05 NS	NS NS			
Halothane Levodopa, 10 mg/kg i.a.	2-5 15	NS NS	<.05 NS	<.05 NS		
Nembutal Levodopa, 10 mg/kg i.a.	2-5 15	<.05 <.05	<.05 <.05	<.05 <.05	<.05 <.05	
Nembutal Levodopa, 20 mg/kg i.a.	2-5 15	<.05 <.05	<.05 <.05	<.05 <.05	<.05 <.05	NS <.05
Animal Group	Time, min	Unanesthetized Levodopa, 10 mg/kg i.a.	Unanesthetized Levodopa, 10 mg/kg i.v.	Unanesthetized Levodopa, 20 mg/kg i.a.	Halothane Levodopa, 10 mg/kg i.a.	Nembutal Levodopa, 10 mg/kg i.a.

* Single factor analysis of variance on repeated measures. Significnce at the $P = 0.05$ level was determined from the least significant difference procedure for pairs of means. All P values $< .05$ are accepted as significant.

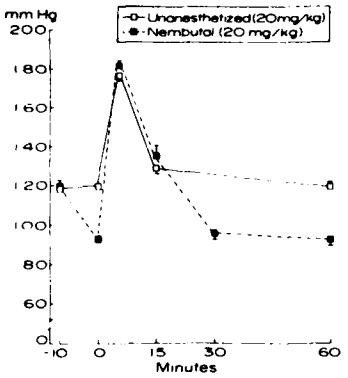

FIG. 3. Mean arterial blood pressure (±SE) of adult female rats after intraarterial levodopa (20 mg/kg) administration.

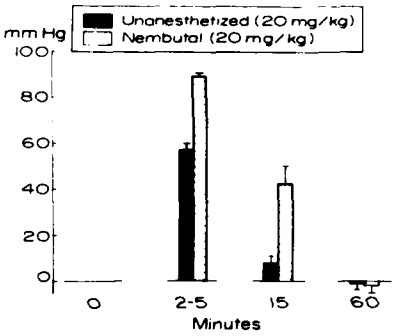

FIG. 4. Increases of the mean arterial blood pressure (±SE) of adult female rats after intra-arterial levodopa (20 mg/kg) administration.

No changes in body weight, body temperature and hematocrit ratio were observed after administration of levodopa.

Discussion. Levodopa therapy causes some derangements of the cardiovascular system (13). Long-term administration of oral levodopa to patients with Parkinson's disease induces orthostatic hypotension in about 30% of the cases (14, 15). The cardiovascular effects of levodopa are associated mainly with dopamine. It has been reported that dopamine stimulates myocardial β-adrenergic receptors (6) leading to increased cardiac contractile forces (16–20) and to an increased cardiac output (21). These effects were used beneficially in the treatment of cardiogenic and noncardiogenic shock (17, 20, 22–24). Dopamine causes hypertension largely by direct interaction with α-adrenergic receptors in vascular smooth muscle replacing (25) or releasing the stores of norepinephrine from sympathetic nerve fibers. Thus dopamine, naturally occurring catecholamine and the immediate precursor of norepinephrine, leads to both α- and β-adrenergic stimulation (26). After inhibition of dopa decarboxylase in peripheral tissues and inhibition or transformation of levodopa into dopamine, the administered levodopa induces hypotension probably due to the effects on the central nervous system (27, 28), presumably on the vasomotor center in the medulla (29). Large amounts of levodopa induce mesenteric, renal (22, 30) and cerebral vasodilation (31). Despite the proven effect of dopamine on alpha receptors, hypertension is rarely observed in patients following oral intake of levodopa. This might be due to the administration of drug in relatively small doses. However, in some patients treated with levodopa, anesthesia causes hypertensive and in others hypotensive responses (32).

Administration of levodopa or of dopamine causes hypertension in dogs (6, 9, 26) and rats (4, 7, 27, 28). The opposite effect, hypotension, is observed in cats (3), guinea pigs, and rabbits (1). These seemingly contradictory data indicate that vascular responses may depend on the animal species, on the administered doses (33), as well as on the route of administration.

Until now, the information about cardiovascular changes induced by levodopa administration was collected on anesthetized animals (several species) or in patients that were undergoing levodopa treatment. There is no information about the effect of levodopa on the circulation of chronically cannulated, unanesthetized, and unrestrained animals. In our work the effect of intra-arterially injected levodopa on arterial blood pressure was studied in unanesthetized, unrestrained, resting rats several weeks after chronic cannulation of the aorta. The levodopa was administered intravascularly in order to observe immediate circulatory effects of this drug unaltered by processes of absorption. It has been reported that orally administered levodopa might be degraded in the liver even before it enters the circulation (34). However, if intra-arterially injected, this drug passes through the blood brain barrier in 15 sec (35).

In our study all unanesthetized rats in-

creased mean arterial blood pressure after intra-arterial administration of levodopa. The increase was greater and appeared to last longer when the levodopa dose was doubled. For the dose of 10 mg/kg of levodopa, the increase of the arterial blood pressure was greater if levodopa was administered intravenously instead of intra-arterially. We believe that this might be associated with the direct action of levodopa on myocardium.

The results on unanesthetized animals were compared to the values obtained in the animals during nembutal or during halothane anesthesia. The later part of this work was done because it is known that anesthesia alters profoundly cardiovascular parameters (10). Furthermore, it was reported that halothane causes myocardial depression and that dopamine either decreases or leaves unchanged the total peripheral resistance in halothane anesthetized animals (24). The dopamine content of the brain is increased after anesthesia, as well as dopamine synthesis and turnover (37).

In our work on nembutal and on halothane anesthetized rats, we found an increased man arterial blood pressure after the injection (ia) of levodopa. The increase was much smaller in halothane anesthesia. This effect might be associated with the depressing action of this anesthetic on the heart and its profound effects on the CNS. The duration of the hypertensive response appeared to be longer in nembutal anesthetized animals that received a larger dose of levodopa. It is interesting to note that the peak value of the mean arterial blood pressure was not much different if 10 or 20 mg/kg of levodopa was given to nembutal anesthetized rats.

Summary. The arterial blood pressure responses in rats after intravascular levodopa administration were measured for the first time with direct methods in unanesthetized, unrestrained animals after full recovery from surgical stress due to the cannulation. The observed results were compared with results collected from anesthetized animals (nembutal or halothane). After levodopa administration, the mean arterial blood pressure of all rats was increased, reaching the peak value 2–5 min later. The animals in halothane anesthesia had a small rise of the mean arterial blood pressure. Doubling the dose of levodopa increased further the hypertensive responses in unanesthetized animals but not in nembutal anesthesia. Percentage-wise, levodopa caused a much greater increase of the mean arterial blood pressure in nembutal groups than in unanesthetized animals. However, the peak values were the same, around 170–180 mmHg, in all nembutal anesthetized animals as in unanesthetized animals that received a double dose of levodopa (20 mg/kg).

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ADDENDUM IV

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Treatment of experimental cerebral infarction in rats with levodopa or with glycerol

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Administration of large amounts of levodopa did not improve survival rates of rats after acute cerebral infarction induced by injection of carbon microspheres. However, when 10% glycerol was used, the number of rats that survived after cerebral infarction was significantly greater than in the control or in the levodopa-treated rats. Combination of levodopa and glycerol therapy also significantly improved the survival rate of infarcted animals. It appears that glycerol alone is the main factor in eliciting this beneficial effect. Pathological findings (gross or microscopic) indicate striking changes in brain tissue after embolization. Development of brain edema of the infarcted left hemisphere corresponded to the type of treatment and to the length of animal survival. Brain-tissue histology indicates that glycerol-treated animals developed less severe edema and had less tissue disruption than control animals. The results suggest that treatment of edema should be one of the primary steps in therapy after acute cerebral infarction.

KEY WORDS • carbon microsphere • edema • levodopa • glycerol • cerebral infarct

THERE is much speculation about the events that occur after cerebral infarction and about the kind of treatment one should adopt. Different experimental models give different results. Clinical data vary from patient to patient and are difficult to evaluate, especially because of differences in time of treatment, and the variability of the causes of infarction and their severity. Although one may expect striking pathophysiological changes induced by the damage to the blood vessels during severe brain ischemia caused by infarction, the picture is further complicated by development of necrosis, edema, and changes in cerebral blood flow. It appears that ischemic injury to neurons begins 10 to 15 minutes after vascular occlusion.[6] Furthermore, it is possible that monoamine neurotransmitters leak into extracellular spaces from the damaged neurons, aggravating further the ischemic insult.[27]

The effect of dopamine on the brain blood flow is not yet well documented, but it appears that it might be of a profound importance. Both an increase and a decrease of neurotransmitter content have been described in experimental brain infarction or in patients suffering from stroke. The precise role of biogenic amines in the formation of brain edema is still unknown.[5] The controlled use of neurotransmitters or of their blockers might

eventually resolve some of the problems associated with cerebral infarction.

The importance of limiting cerebral edema as soon as possible after a cerebrovascular accident is well known.[19] The interplay of edema with other pathophysiological changes occurring at the same time is poorly understood.

The aim of this work was to study, in controlled experiments, the effect of levodopa and glycerol on recovery from cerebral infarction in rats after embolization with carbon microspheres.

Materials and Methods

Ninety-nine adult female Sprague-Dawley rats, weighing 212 ± 21 gm (SD), were used in the experiments. The technique of induction of cerebral embolization with carbon microspheres described by Kogure, *et al.*,[9] was adapted for our work. The intracarotid injection of microspheres is a reproducible method for inducement of cerebral infarction in small laboratory animals.[9,20] Under Fluothane (halothane) anesthesia, one polyethylene (PE 10) cannula was inserted through the left common carotid artery with its tip floating in the aortic arch,[16] and its free end exteriorized on the back of the animal's neck. This procedure lasted 5 minutes. The cephalic part of the left internal carotid artery was then temporarily cannulated with PE 50 polyethylene tubing. Immediately after the placement of the PE 50 cannula, the brain was embolized with carbon microspheres (35 ± 5 μ in diameter, injected through the PE 50 cannula) suspended in rat's plasma (5 mg/ml). Total injected volume was approximately 0.085 ml (0.43 mg microspheres) given in the form of a bolus. Aggregation of microspheres in plasma was avoided by the use of a magnetic stirrer before injection, and agitation with two small brass bearings in the tuberculin syringe during injection. The cannula was not rinsed because additional injection could displace already lodged microspheres. As soon as the microspheres were injected, the PE 50 cannula was removed and the left carotid artery peripherally ligated. The wound was sutured and anesthesia was discontinued. Five minutes later the embolized animals received drug therapy (levodopa, 20 mg/kg intra-arterially, dissolved in saline 10 mg/ml; and/or glycerol, 1 gm/kg, dissolved in saline as a 10% solution) through the aortic PE 10 cannula. Control animals received the same volume of saline intra-arterially. Repeated administration of agents was given through the same chronic aortic cannula.

Statistical treatment consisted of calculating chi-square and probability values from contingency tables prepared to evaluate homogeneity between control animals, and association between treatment and recovery. The null hypothesis that recovery was independent of treatment was rejected at the $p < 0.05$ level.

The animals used in this study were randomly divided into five groups as follows:

Group 1: Control Group. This group consisted of 22 animals. After embolization with microspheres, 11 animals received a single saline administration (2 ml/kg, which corresponded to the volume of levodopa received by the animals in Group 2). Another 11 animals received saline twice a day (8 ml/kg, which corresponded to the volume of glycerol received by the animals in Group 4). An additional group of seven animals was sacrificed for histological examination 48 hours after brain embolization.

Group 2: Single Levodopa Administration. The 22 animals in Group 2 received a single injection of 20 mg/kg levodopa (dissolved in saline, 10 mg/ml) after cerebral infarction.

Group 3: Repeated Levodopa Administration. The 10 animals in Group 3 received 20 mg/kg levodopa twice a day for 1 week.

Group 4: Repeated Glycerol Administration. The 22 animals in Group 4 received 10% v/v glycerol (1 gm/kg, dissolved in saline) twice a day for 1 week. An additional group of six animals with this treatment was sacrificed 48 hours after embolization for histological study.

Group 5: Repeated Glycerol and Levodopa Administration. The 10 animals in Group 5 received 20 mg/kg levodopa and 10% glycerol twice a day for 1 week.

Infarcted brains of animals that died 2 days after infarction, or were sacrificed (on Day 2 or Day 15) were studied histologically.

Results

Group 1: Control Group

Only 23% of the control animals survived cerebral infarction. The observed hetero-

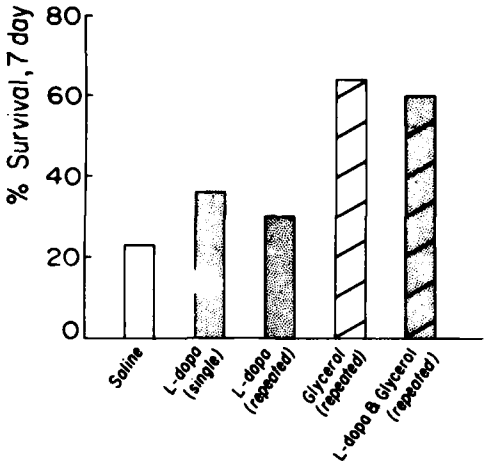

FIG. 1. Survival of rats after cerebral infarction induced by injection of carbon microspheres.

geneity chi-square values indicated that the data were homogeneous and there was no difference in survival between the animals that received single administration of saline and those that received repeated saline administrations. For this reason all control animals were pooled as one group (Fig. 1, Table 1).

Eleven animals in the control group died or were sacrificed within 48 hours after embolization. On gross examination the left brain hemispheres of most of these animals were enlarged and "boggy," suggesting moderate-to-severe edema. Microscopic examination of coronal sections of the cerebrum and cerebellum in each rat revealed that numerous microspheres were present in the left cerebral hemisphere, the side of microsphere injection. Only an occasional microsphere was observed in the opposite hemisphere, usually within meningeal vessels. No foci of edema or infarction were noted in the right hemispheres. An occasional microsphere was seen in the cerebellum or brain stem, but without tissue changes. Microscopic zones of cerebral infarction and early cavity formation were demonstrable in the injected left hemispheres, usually in close proximity to microspheres (Fig. 2 *left*). Adjacent to microspheres, large foci of severe reactive edema were observed. In some areas the tissue distortion and fragmentation was suf-

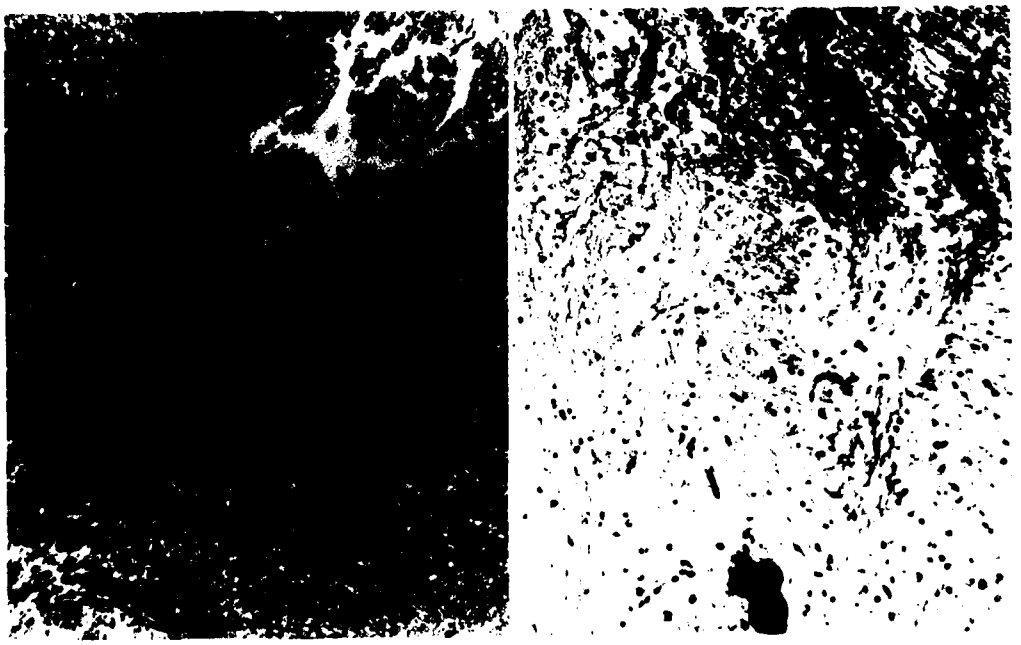

FIG. 2. Photomicrographs from Group I rats. H & E, × 100. *Left:* An area of infarction and early cavity formation (above) and microspheres (below) can be seen. Note the generalized edema. *Right* Intravascular microspheres (below) are associated with marked distortion of neuropil by edema.

TABLE 1

Survival of rats after controlled cerebral infarction (microspheres) treated with levodopa and/or glycerol

Treatment	No. of Rats	Survival, Day							No. of Rats Survived
		1	2	3	4	5	6	7	
saline	22	19	9	5	5	5	5	5	5
levodopa (single)	22	19	10	9	9	9	8	8	8
levodopa (repeated)	10	9	5	3	3	3	3	3	3
glycerol (repeated)	22	19	14	14	14	14	14	14	14
glycerol + levodopa (repeated)	10	9	6	6	6	6	6	6	6

ficiently advanced to indicate early infarction (Fig. 2 *right*).

Group 2: Single Levodopa Administration

Of the 22 animals in this group, 36% survived cerebral infarction. There was no significant difference in survival between this group and the control animals (Fig. 1, Table 1).

Group 3: Repeated Levodopa Administration

Of the 10 animals in Group 3, 30% survived cerebral infarction. There was no significant difference in survival between this group and the control group (Fig. 1, Table 1).

The four animals in this group that died approximately 48 hours after embolization showed on gross examination evidence of boggy enlargement of the left brain hemisphere. Foci of hemorrhage were noted in three animals. Microscopically, brain tissue of animals in this group appeared similar to the brain tissue in control animals. Microscopic areas of cavity formation in the periventricular white matter were also demonstrated. Marked edema was present in the gray matter and in the subjacent white matter (Fig. 3).

Group 4: Repeated Glycerol Administration

Of the 22 animals in this group, 64% survived cerebral infarction. This represents a highly significant difference ($p < 0.01$) in survival between this and the control group (Fig. 1, Table 1).

Six animals were sacrificed 48 hours after embolization with microspheres. This was done to compare pathological findings with the control animals sacrificed at the same time. Brain tissue of animals that received repeated glycerol administration shows less severity of edema and less tissue disruption than brain tissue of control animals. Foci of edema are more circumscribed. Severe edema

FIG. 3. Photomicrograph from a Group 3 rat. Extensive edema is present in the ganglion cell layer and subjacent white matter (left). H & E, × 100.

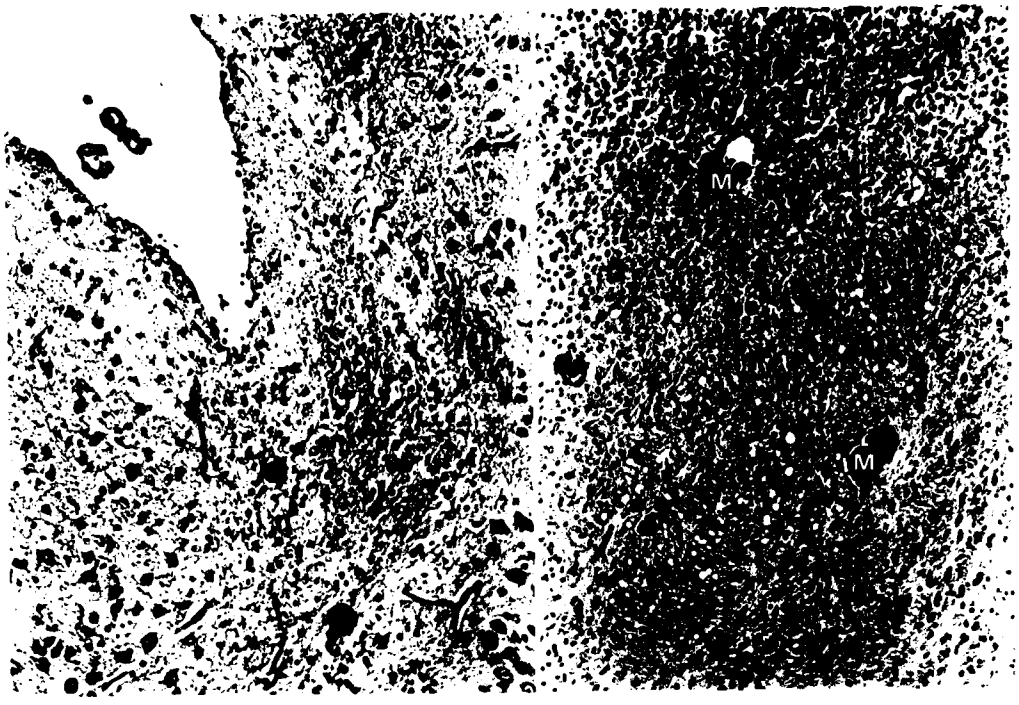

FIG. 4. Photomicrographs from Group 4 rats. *Left:* Focal edema and cavity formation (above) are seen in proximity to an intravascular microsphere (below). Note the normal neurons (right and below left) lying adjacent to the walls of the cavity and to the area of residual edema. H & E, × 100. *Right:* There is secondary cellular infiltration, including "gitter" cells (G), in a focal zone of cerebral infarction adjacent to microspheres (M). There is a sharp demarcation from the brain tissue containing normal neurons (N) adjacent to this infarcted area. H & E, × 70.

was rarely seen. Glycerol did not prevent cavity formation nor infarction. Occasional microspheres were seen in the right hemisphere, cerebellum, or brain stem, but without tissue infarction.

On Day 15 after embolization, 11 animals treated with repeated glycerol administration were sacrificed. More than half of the animals displayed evidence of infarction or cyst formation on gross examination of coronal sections. Microscopically, there was well defined cavity formation in many of these brain tissues. There was a sharp demarcation between edematous zones, which were largely confined to the immediate vicinity of microspheres, and the surrounding brain, which appeared mildly reactive but contained normal neurons (Fig. 4 *left*). There was no evidence of residual edema in most of the normal brain tissues of the microsphere-injected hemispheres. In general, brains of glycerol-treated animals revealed a striking decrease in the amount of edema and in the size of infarcted areas where cavity formation was present. The histological changes appeared to be focal and well demarcated, with no generalized brain edema in the injected hemispheres in contrast to the brains of animals in the control group. The numbers of microspheres were approximately the same in both groups, but there was a reduction in the number and size of cysts produced in the treated group. In some areas, in which infarction had occurred, numerous "gitter" cells could be demonstrated. These focal areas were usually confined to the vicinity of the microspheres (Fig. 4 *right*). The contralateral hemispheres showed no evidence of edema or infarction.

Group 5: Repeated Glycerol and Levodopa Administration

Of the 10 animals in Group 5, 60% survived. This represents a significant difference ($p < 0.05$) in survival between this and the control group (Fig. 1, Table 1).

FIG. 5. Photomicrograph from a Group 5 rat. Large cavity (above) after infarction, surrounded by cellular infiltration in the vicinity of microspheres (H & E, × 25).

Four animals in this group were sacrificed 15 days after embolization. In microscopic sections, cavity formation could be demonstrated adjacent to microspheres in several areas. Cellular infiltration was present in areas of previous ischemia (Fig. 5), but was not generalized. Foci of edema adjacent to intravascular microspheres could be demonstrated, but were sharply localized. Surrounding brain showed no abnormalities. In general, cavity formation was not prevented by this regimen, but foci of edema were markedly reduced and were sharply circumscribed. There was less distortion of brain by residual edema.

In all animal groups the highest mortality was observed on Day 2. All glycerol-treated animals survived after Day 2, while in other groups mortality continued (Table 1).

Discussion

At present it is not clear what is the preferable therapy after cerebral infarction.[1] Increase of arterial blood pressure, decrease of blood viscosity,[22,23] hypothermia,[15,16,19] and reduction of brain edema,[7,11,12] have been suggested as the best treatment regimens. However, because of differences in experimental procedures and because of the great variety of conditions in patients with cerebral infarction, the best therapeutic procedures are still not established. Although many pathophysiological changes are occurring after brain infarction, it is possible that there is only one major cause leading to morbidity and mortality of the experimental animals or patients, while other changes are only accompanying the main process. If this were true, a specific drug could bring profound amelioration and better recovery after cerebral infarction.

Because of their vasoactive effects,[8,24] catecholamines probably play a role in changes induced after cerebral infarction, but the relationship between cerebral infarction and catecholamines is still not well understood.[3,10,13,26,27] After cerebral infarction, edema (to a certain degree) always develops in various model systems.[2,4,9,14,23] Severe edema appears to be a major cause of death in patients,[7,22] and experimental animals.[22] Most patients with cerebral arterial occlusion have a combination of vasogenic and cytotoxic edema.[5] In human cerebral infarction, edema might be the consequence of developing necrosis.[14]

It has been shown that osmotherapy brings some improvement from brain edema.[17,21] Glycerol, a hyperosmolar agent, has the ability to reduce the effects of cerebral edema.[4,11,12] Gilsanz, *et al.*,[7] described in patients with acute cerebral infarction a significant improvement after intravenous treatment with 10% glycerol for 6 days, as compared with dexamethasone treatment. Although dexamethasone has some therapeutic value in the treatment of vasogenic edema associated with brain tumors and brain abscesses, its effectiveness in the case of acute infarction has not been well established, and the literature regarding its use in stroke has been inconsistent and controversial.[6]

Conclusions

In this work, during the acute stages of experimentally induced cerebral infarction, we studied the effect of levodopa, a monoamine

that rapidly passes the blood-brain barrier,[26] and glycerol, an agent that reduces brain edema because of its hyperosmolarity. We also studied the effect of both drugs when combined to see if such a therapy is more beneficial. Our results indicate that glycerol-treated animals had a decreased mortality. This is probably because of the hyperosmolitic property of glycerol, since pathological findings demonstrated that repeated treatment with glycerol produced a marked decrease in brain edema, confining the edema to focal areas adjacent to microspheres. Infarction resulting in cavity formation was not prevented, but edema and cellular reaction around these zones of infarction were markedly reduced in size and were sharply circumscribed. Pathological changes of brain tissue in animals treated with levodopa and glycerol were similar to those found in only glycerol-treated animals. Animals in the control group had generalized edema of ipsilateral hemisphere, and a short survival time. Results after levodopa therapy only were not different from results in the control animals. It appears, therefore, that the use of glycerol treatment immediately, and repeatedly, after experimental infarction with carbon microspheres reduces the extent of brain edema and cellular reaction.

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