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CHANGES IN THE OXYGEN-HEMOGLOBIN DISSOCIATION CURVE AND TIME OF--ETC(U)
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CHANGES IN THE OXYGEN-HEMOGLOBIN DISSOCIATION CURVE AND TIME OF USEFUL FUNCTION AT HYPOBARIC PRESSURES IN RATS AFTER CHRONIC ORAL ADMINISTRATION OF PROPRANOLOL

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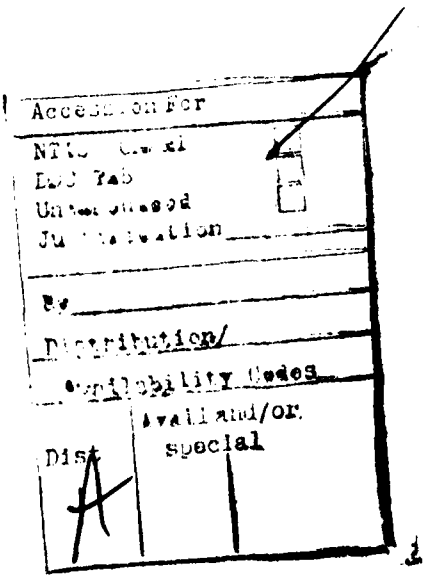

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16. Abstract

Twenty rats were given propranolol, 1.8 mg/kg body weight, by mouth for 12 days. On day 13, these and 20 control rats were tested for time of useful function (TUF) after rapid decompression in a hypobaric chamber. The criterion for TUF was the animal's ability to remain upright in a motor-driven exercise wheel. The decompression profile was achieved by rapidly reducing the pressure to a level equivalent to an altitude of 34,000 ft (10,363 m), holding for 1 min, then recompressing and holding at 29,000 ft (8,839 m) for the remainder of the test. On day 13 the propranolol-treated rats had a significant shift to the right in the oxygen-hemoglobin dissociation curve (ODC); their average p_{50} was about 3.5 torr greater than that of control animals. Although shifts to the right in the ODC normally occur during adaptation to altitudes, this was of no advantage to the drug-treated group, which had an average TUF of only about 170 s (approximately 50 s less than that of control rats). This finding is consistent with the results of Eaton et al., who found that rats with left-from-normal shifts in the ODC survived longer than did control rats at altitudes approximately equal to those used in the present study. In other experiments we showed that one metabolite of propranolol, 4-hydroxypropranolol, can produce rightward shifts in the ODC. This result may help explain why chronic administration of the drug causes such shifts, though its concentration in the blood is far less than that required to produce the effect in vitro. Decrements in the TUF seen in these and other (FAA Office of Aviation Medicine Report No. AM-79-10) experiments were probably the result of several mechanisms, including shifts in the ODC, central depression, and cardiovascular effects.

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CHANGES IN THE OXYGEN-HEMOGLOBIN DISSOCIATION CURVE AND TIME OF USEFUL FUNCTION AT HYPOBARIC PRESSURES IN RATS AFTER CHRONIC ORAL ADMINISTRATION OF PROPRANOLOL

Propranolol is currently one of the most widely used β-sympatholytic drugs; a recent survey indicates that over 2 million patients receive it for a variety of conditions, including hypertension, ischemic heart disease, and cardiac arrhythmias (Table I). Because of its effectiveness in relieving these conditions, on which aeromedical disqualifications can be based, the Federal Aviation Administration has an interest in evaluating the drug for use by airmen.

One interesting property of propranolol that bears on its use by airmen is its ability to produce shifts in the oxygen-hemoglobin dissociation curve (ODC); no other β-adrenergic blocking agent so far examined possesses this property. The effect is produced in vitro at concentrations approaching 26,000 ng/mL, but lower concentrations do not produce shifts in the ODC in vivo, except in patients in whom these lower levels (less than 400 ng/mL) are maintained for a considerable number of days.

In 1979 (9) we reported significant decreases in the time of useful function (TUF) of rats during the first hour following single intraperitoneal injections of the drug, but we did not find any shifts in the ODC of these animals; plasma concentrations of propranolol were on the order of 200 ng/mL. Although these experiments contributed to our knowledge of the drug's acute effects, they did not apply to the chronic, more clinically appropriate effects. This present report contains such information, as well as data that may explain the shift in ODC that occurs after long-term therapy.

METHODS

Measurement of Oxygen-Hemoglobin Dissociation: Blood samples were taken from the inferior vena cava or tail vein of rats or from the antecubital vein of human donors. In experiments involving the administration of propranolol to rats, samples of heparinized whole blood were used. For in vitro tests, in which the drug was added to suspensions of erythrocytes, the suspending medium was Krebs-Henseleit buffer. Regardless of the suspension medium (buffer or plasma), the gas mixtures used were the same: 25% O_2/5.6% CO_2/69.4% N_2. The ODC was measured in the Hem-O-Scan oxygen dissociation analyzer (American Instrument Company, Silver Spring, MD) using program A of the instrument.

Drugs: Propranolol and 4-hydroxypropranolol were gifts from the Medical Department of Ayerst Laboratories, 685 Third Avenue, New York, NY 10017.

Chronic Experiment: Approximately 50 male albino rats (Charles River Breeding Laboratories, Inc., Wilmington, MA 01887) were received into our animal facility and allowed to stabilize for 10 days on an ad lib diet of

TABLE I. PROPRANOLOL (INDERAL®). FREQUENCY OF USE, BY AGE AND INDICATION IN THE CONTINENTAL UNITED STATES, SEPTEMBER 1978 THROUGH AUGUST 1979.*

	Age Group							
	1 - 19	20 - 29	30 - 39	40 - 49	50 - 59	60 - 64	65 & Over	Total
Indication								
Hypertension								
% of Use	.3	2.4	5.4	17.4	28.7	15.5	30.3	100.0%
Number of Patients (000's)	3	22	49	159	263	142	277	915
Angina								
% of Use	.1	.3	1.5	10.8	22.9	17.5	46.9	100.0%
Number of Patients (000's)	1	3	13	92	194	149	398	850
Arrhythmia								
% of Use	.2	4.4	7.3	8.7	17.7	11.9	49.8	100.0%
Number of Patients (000's)	1	15	26	30	62	42	174	350
Migraine								
% of Use	1.3	21.1	29.4	14.7	24.8	3.8	4.9	100.0%
Number of Patients (000's)	**	8	12	6	10	1	2	40
All Other								
% of Use	.2	2.0	4.3	11.0	23.7	14.5	44.3	100.0%
Number of Patients (000's)	***	2	4	10	22	14	42	95
Grand Total								
% of Use	.2	2.2	4.7	12.6	24.4	14.9	41.0	100.0%
Number of Patients (000's)	5	50	106	283	549	335	922	2,250

*Source: National Disease and Therapeutic Index; IMS America, Inc. (Courtesy of Ayerst Laboratories).
**Less than 1,000 (520)
***Less than 1,000 (190)

Purina Laboratory Chow and water containing sodium sulfathiazole (Sulfa-Lite, Bio-Centric Laboratories, St. Joseph, MO; dose rate: 10 g of the product per gallon of water). At the end of this time 40 of these rats with body weights of 202 to 224 g were selected for the experiment. The experimental group of 20 rats received daily, by gastric intubation, 1.8 mg (free base) propranolol per kg of body weight. The drug, dissolved in 0.9% NaCl solution with a minimum of citric acid, was of such concentration as to require an administered volume of 1.0 mL of the solution per kg of body weight. The 20 control rats received the same volume dose of pure saline.

Because the number of measurements of the ODC that could be accomplished in any one day was limited, the rats were divided into subgroups and the experimental protocol was arranged as follows: Paired groups of five experimental and five control animals began the experiment on each of 4 consecutive days; thus the first two groups received their first dose of drug or saline on day 1, the second pair of groups on day 2, another on day 3, and the last set on day 4. Final doses were given on days 12, 13, 14, and 15, and the altitude test was administered on days 13, 14, 15, and 16, respectively.

Altitude Test: Twenty-four hours after the final dose was given, the TUF was measured for each rat in the hypobaric chamber. A rapid decompression profile was employed; rats were tested in pairs with one control animal and one experimental animal placed in a double-compartmented exercise wheel (Figure 1 in reference 9) during each trial. The rapid decompression profile and requirements for physical exertion were identical to those described in reference 9 except that, for technical reasons related to hypobaric chamber operation, the rats exercised only 25 s, rather than 1 min before decompression. Criteria for assessing useful function were identical to those used previously; the endpoint was recorded as the time elapsing from initial decompression until the animal could no longer walk in the rotating wheel. After this time had been recorded the chamber was recompressed and the animals were removed. The tip of the tail was then cut, a small sample of blood was mixed with heparin, and the sample was immediately refrigerated for later measurement of the ODC. Each rat was then anesthetized with diethyl ether, and a blood sample of approximately 5 mL was removed from the inferior vena cava, mixed with heparin, cooled in an ice/water bath, and centrifuged at 5°C. Two milliliters of plasma were mixed immediately with 30 mg of sodium metabisulfite, frozen rapidly, and stored at -20°C for later measurement of propranolol and 4-hydroxypropranolol by high-performance liquid chromatography.

In Vitro Tests of Propranolol and 4-Hydroxypropranolol: Stock solutions of the hydrochloride salts of these compounds were made using 0.9% NaCl solution as the diluent. These stock solutions contained 3.47×10^{-2} moles of the drug per liter of solution; they were prepared on the week of the tests and kept under refrigeration. On the day of the tests each stock solution was further diluted in Krebs-Henseleit buffer (see Table II) to a concentration that, when diluted an additional 50%, would yield in final suspensions of erythrocytes concentrations of $1.735, 0.868$, and 0.434×10^{-4} M.

TABLE II. KREBS-HENSELEIT BUFFER. THIS BUFFER IS ESSENTIALLY THAT DESCRIBED BY H. A. KREBS AND K. HENSELEIT, HOPPE-SEYLER'S ZEITSCHRIFT FÜR PHYSIOLOGISCHE CHEMIE 210:33, 1932. IT IS MADE FROM SIX STOCK SOLUTIONS IN DEIONIZED WATER. FINAL BUFFER IS GASSED WITH 5.6% CO_2 JUST BEFORE USE. Courtesy, American Instrument Company.

Stock #	Composition	Ratio In Final Buffer Solution
1. NaCl	0.154 M	100
2. KCl	0.154 M	4
3. $CaCl_2$	0.110 M	3
4. KH_2PO_4	0.154 M	1
5. $MgSO_4.7H_2O$	0.154 M	1
6. $NaHCO_3$	0.154 M (Gas with 100% CO_2; Store under CO_2)	21

Human or rat venous blood was drawn into new plastic syringes, immediately mixed with heparin, and placed in an ice bath. Within 20 min the sample was centrifuged and the cells were washed twice with an equal volume of cold buffer. The cells were centrifuged a third time, diluted with one-half volume of buffer, and placed in a refrigerator at 5°C until used in the tests.

For each test the buffer-suspended cells were well mixed and 500-µL portions of the suspension were mixed with 250 µL of drug solution in small vials. Each vial was then returned to the refrigerator. Twenty minutes prior to measurement of the ODC each vial was removed from the refrigerator and allowed to warm to room temperature. The Hem-O-Scan device was set to program A; the sample was inserted into the chamber and allowed to adjust for 20 min at 37°C in an atmosphere containing 5.6% CO_2 and 94.4% N_2 before the program was started. A vial containing cells suspended in pure buffer provided control samples; these were tested before and after each trial, and all data were discarded if the two control runs did not agree within 2 torr.

RESULTS

Chronic Effects of Propranolol: There was little difficulty in administering either the drug or the control saline solution. Aside from the temporary appearance of loose stools in most of the experimental group about the 4th day, there was little apparent response from the drug. Table III presents data on the body weights on the 4th and 12th days of the experiment. Average weights (and standard deviations) of control and experimental animals

TABLE III. CHANGES IN BODY WEIGHT OF RATS DURING TREATMENT WITH PROPRANOLOL OR CONTROL SALINE SOLUTION. PAIRED GROUPS.

(See text for details. Average of 5 rats (± standard deviation).)

Group	N	DAY 4	DAY 12
A*	5	234.4 (9.0)	269.8 (7.9)
B**	5	232.2 (11.6)	274.0 (12.9)
C*	5	245.4 (11.6)	283.8 (22.9)
D**	5	248.4 (11.5)	294.8 (21.4)
E*	5	245.8 (11.8)	280.0 (22.2)
F**	5	246.2 (18.9)	287.6 (25.9)
G*	5	259.8 (6.6)	294.2 (8.0)
H**	5	255.0 (12.9)	289.4 (12.2)

*Saline Control
**Propranolol
Note: Starting (day 1) weights for 20 control and 20 experimental animals averaged 211.6 (5.2) and 211.0 (6.2) g, respectively.

on the 4th day were 246.35 (13.01) and 245.45 (15.47) g respectively; on the 12th day average weights were 281.95 (17.85) and 286.45 (19.3) g. Differences between the means were not statistically significant by the unpaired t-test.

Although TUF data are valid for this experiment, they cannot be compared directly to those presented in the earlier report because of technical differences in the rapid decompression protocol (see METHODS: Altitude Test). As in the earlier study (9), propranolol-treated rats lost useful function sooner than did control animals, but average differences were not statistically significant. Note, however, that standard deviations of the data are much larger than those seen in earlier data (see Table IV).

On the 4th day some attempts were made to measure representative ODCs (one rat in each of the eight groups), but technical analytical problems prevented exact measurements except in a few animals. In these few experimental animals there was a tendency for a rightward shift in the curve.

By the 13th day all technical problems had been remedied and accurate measurements were possible. At this time the mean p_{50} of the propranolol group was approximately 3 torr greater than that of control animals, and this difference, presented in Table IV, was significant at the 0.05 probability level by the unpaired t-test.

TABLE IV. TIME OF USEFUL FUNCTION (TUF) AND OXYGEN-HEMOGLOBIN DISSOCIATION (ODC) IN PROPRANOLOL-TREATED AND CONTROL RATS AFTER RAPID DECOMPRESSION.

Average values (± standard deviation).

Group*	TUF (Seconds) (n=20)	ODC (Torr)** (n=15)
C	219.45 (129.53)	40.3 (4.3)
P	169.9 (80.9)	43.7 (4.6)***

*C= Saline Controls; P= 1.8 mg propranolol per day for 12 days.

**P_{50}: partial pressure of oxygen resulting in 50% saturation of hemoglobin.

***Difference between control and experimental P_{50} was significant at the 0.05 level. TUF difference was not significant.

Because several reports have referred to changes in erythrocyte morphology in patients treated with the drug (6,13,14), we prepared blood smears of all samples taken from control and experimental animals. These were stained with Wright's stain and examined under an oil immersion lens for abnormal cells. There were no consistent differences between the morphology of control and experimental cells. A slight degree of anisocytosis was seen in both groups.

In Vitro Experiments: Propranolol produced typical shifts in the ODC at all concentrations tested; because of a shortage of gas mixtures no trials of 4-hydroxypropranolol were made with human cells; all available supplies were expended in several trials with rat erythrocytes. In all of these trials both drugs produced rightward shifts in the ODC of approximately 3 torr. In one test involving whole human blood, propranolol did not produce a shift, even at a concentration of 1.7×10^{-4} M. The blood donor in this test was a smoker who consumed approximately 1.5 packs of cigarettes per day. His P_{50} (control) was 23.5 torr, typical of those with a significant level of carboxyhemoglobin due to smoking.

DISCUSSION

Since the mid-1960s β-sympatholytic agents have found considerable use in the treatment of hypertension, ischemic heart disease, and other conditions (Table I). Propranolol, unlike some other drugs in this category, blocks both β_1 and β_2 (myocardial and vascular) receptors. It has been cited as one of the most effective and one of the safest drugs of the type available (10). Without question, propranolol's sympatholytic activity is of paramount importance, but there are other properties that may play a part (see, for

example, the review by Kaverina and Chumburidze (10)). One unusual property of propranolol is its ability to cause rightward shifts in the ODC, a phenomenon first reported by Pendleton et al. of the Smith Kline and French Laboratories (13,14). An absence of the effect with other β-blockers indicated that adrenergic mechanisms were not involved, and 2,3-diphosphoglycerate, a metabolite known to produce rightward shifts in the ODC, was not increased in erythrocytes exposed to propranolol. The authors also found changes in erythrocyte morphology and failed to demonstrate any ODC effect in cell-free solutions of hemoglobin.

Agostini et al. (1) showed that propranolol causes a leakage of cellular K^+, an effect known to decrease intracellular pH and consequently increase the p_{50} of oxygen-hemoglobin dissociation. Fortier et al. (6) demonstrated that the drug's influence on both K^+ and ODC could not occur in the absence of Ca^{++} and that propranolol caused an increased incorporation of this ion into the cells. This finding is apparently a result of the ubiquitous "Gardos effect" (7,15).

Despite the ability of propranolol to produce changes in the ODC in vitro at concentrations above 10^{-4} M, Lichtman et al. (11) were not able to demonstrate the effect in human subjects for up to 24 h following oral doses of up to 40 mg. These authors attributed the discrepancy to the fact that blood concentrations in those taking the drug are only 1/100 of the required concentration. Their conclusion was not supported by Schrumpf et al. (16), who observed shifts in the ODC of 12 patients with angina pectoris who had been under medication for at least 3 months.

Thus, propranolol administered over long periods can produce the ODC effect, even in patients who receive only 30 mg/day (patient #1; Schrumpf et al.) and whose serum levels of the drug remain below that concentration required for the acute in vitro effect (5,13,14). Two explanations for this difference between acute and chronic effects are possible: Either (i) with chronic administration the drug becomes more associated with the erythrocyte membrane or in some way slowly alters the membrane, or (ii) the metabolites of propranolol are comparably effective in shifting the ODC and, as their concentrations increase during chronic medication, some levels of metabolite(s) are reached that, in sum, are sufficient to shift the ODC. One of our observations supports the latter possibility. The metabolite 4-hydroxypropranolol was found to produce shifts in the ODC at concentrations comparable to those that were effective for the parent drug. Other metabolites of propranolol, not too different from it in structure (5,12), may also be effective in this way. It is interesting that 4-hydroxypropranolol is almost as effective a β-blocking agent as the parent compound, and is pharmacologically distinguishable from it only by virtue of its initial sympathomimetic properties (5). This weak stimulatory activity of 4-hydroxypropranolol would not be detrimental. The parent, having no stimulatory properties, would serve to block those of the metabolite, but both compounds could contribute toward the total blockade of β-receptors.

Although the decrement of TUF due to propranolol seen in the present experiments was not statistically significant (probably due to a high variance), nevertheless this decrement, producible 24 h following the last of 12 daily doses of 1.8 mg/kg, was in the same direction as that of our earlier observations following single acute doses of propranolol. A shift to the right in the ODC might have been expected to be an advantage during altitude exposure. Rightward shifts in the ODC occur in man during the first 36 h of altitude exposure, and this is considered generally to be favorable to survival. Eaton, Skelton, and Berger (3), on the other hand, point out that this may not be the case when the shift impinges on the oxygenation of hemoglobin in the lungs. Inspection of the ODC of man, for example, shows that the blood will become only 95% saturated at a pO_2 of 70 torr (p_{50}=26 torr). Assuming a p_{H_2O} of 47 torr and a p_{CO_2} of 40 torr, the total atmospheric pressure necessary to result in an alveolar p_{O_2} of 70 torr is: $p_B = ((p_{O_2}+p_{CO_2})/(.21))+p_{H_2O}$ = 571 torr, equivalent to an altitude of approximately 7,700 ft (2,347 m). With a rightward shift the 95% saturation point becomes greater than 70 torr, and the threshold altitude at which significant decrements in oxygen loading occur is lowered. Thus, although a rightward shift is usually an appropriate physiological response to hypoxemia, it is not appropriate at extreme altitude. Indeed, Eaton's group showed that rats treated with NaOCN to shift their ODCs from a normal p_{50} of about 37 torr to one of approximately 21 torr survived for over 90 min at a pressure equivalent to 28,000 ft (8,534 m), while only half of the control animals survived the 50th min of exposure (3).

The mechanism by which propranolol reduced the TUF in our experiments is unknown, but certain reports in the medical literature indicate that more than one factor may be involved. Eaton et al. (3) found that, in the altitude-exposed rat, death is preceded by progressive bradycardia. It is not proven that this bradycardia is contributory to hypoxia-induced morbidity, but the possibility seems likely. Fitzgerald (5) points out that bradycardia is a side effect of propranolol therapy. The drug also produces effects in the central nervous system. Bainbridge and Greenwood (2) reported that propranolol produces a tranquilizing effect in rats and that this action is also produced by the dextroisomer, which does not possess β-blocking activity. Hemmingsen et al. (8) showed that propranolol at a dose of 2 mg/kg blocks the increase in the cerebral oxygen consumption stimulated by CO_2. It also reduces the increase in cerebral blood flow caused by CO_2.

Although the changes in TUF seen in the present series do not qualify statistically, they are nevertheless consistent with earlier findings (9); moreover, they represent the effects of a chronic treatment that had been discontinued 24 h prior to the TUF trials. We conclude, from the present and previous studies: (i) Propranolol reduces the time of useful function of rats at high altitude, at least under the conditions of these studies. (ii) The mechanisms responsible for this reduction of TUF may be manifold, perhaps involving β-blocking as well as nonadrenergic actions of the drug. Both cerebral and cardiac mechanisms may play a part, and, in the case of chronically treated rats, a shift to the right in the oxygen-hemoglobin dissociation curve may also contribute. (iii) Observations that propranolol, at moderate blood levels, produces shifts in the ODC of patients under long-term therapy but not

in those who have recently begun therapy, are possibly explained by our finding that the metabolite 4-hydroxypropranolol is equally effective in producing the shift. Thus, the sum of the levels of propranolol and its metabolite(s) may equal a total concentration necessary to produce the effect. (iv) Extrapolations of these results to human beings under propranolol medication should be made with caution, especially with regard to quantitative aspects of dose and altitude tolerance; the p_{50} of rat blood is at least 10 torr to the right of that of normal human blood, and this alone should make a significant difference in altitude tolerances of the two species.

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