THE GANGLIONIC BLOCKING PROPERTIES OF THE CHOLINESTERASE REACTI--ETC(U)

JAN 78 P M LUNDY; D H MCKAY
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OF THE CHOLINESTERASE REACTIVATOR HS-6 (U)

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P.M. Lundy & D.H. McKay

PROJECT NO. PCN 13D23

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ABSTRACT

Following i.v. administration of 30 mg/kg of the cholinesterase reactivator HS-6, blood pressure fell (up to 50 mm/Hg) and maximal blood levels of HS-6 reached 242 μg/ml. HS-6 attenuated the pressor response resulting from carotid occlusion and the depressor effect of vagal stimulation. Doses of HS-6 below those tested therapeutically against soman in different animal species (3.59-10.77 mg/kg) progressively blocked the ganglion stimulating effects of nicotine and dimethylphenylpiperazinium but not those following adrenaline, a pattern similar to that produced by hexamethonium but only 1/84 as potent. HS-6, like hexamethonium and mecamylamine, progressively blocked the contraction of the nictitating membrane of the cat resulting from pre-ganglionic stimulation.

The results indicate that HS-6 possesses ganglion blocking properties at doses likely to have any value therapeutically in soman poisoning. The ganglion blocking properties of the drug may be a factor in the beneficial effects of HS-6.
INTRODUCTION

Therapy for individuals poisoned with organophosphorus cholinesterase inhibitors includes the use of cholinesterase reactivators with one or more oxime groups. Therapy with anticholinergic compounds in combination with oximes such as obidoxim (Toxogonin\textsuperscript{R}), TMB-4 or pralidoxime chloride (2-PAM) has been shown to be effective in saving animals from multiple LD\textsubscript{50} doses of various organophosphate compounds (review see Hobbiger, 1963).

Some organophosphates such as soman (o-pinacolyl-methylphosphonofluoridate) produce an inhibited cholinesterase which rapidly ages, rendering the inhibited enzyme refractory to reactivation by the oximes mentioned previously. However, the use of cholinolytics with oximes such as HS-6, 

\[ [(2-hydroxyimino-methyl)-pyridinium-(1)-methyl]-[3-carbamyl]-pyridinium-(1) methyl]-ether dichloride \]

(Figure 1), have been shown to produce success in protecting animals from several LD\textsubscript{50} of soman, but the mechanism of this protective effect is not yet clear (Oldiges and Schoene, 1970; Schenk et al., 1976; Wolthuis et al., 1976).

The pharmacological effects of HS-6 have not been well studied and indeed the pharmacology of the classical reactivators is also less than adequately understood. With the increasing possibility that compounds similar
[\text{(2-hydroxyimino-methyl)-pyridinium-(1)-methyl}]-\text{[\text{(3-carbamyl)-pyridinium-(1)-methyl}]}-\text{ether dichloride}

\text{FIGURE 1: The chemical structure of HS-6.}
to HS-6 could be of significant benefit in organophosphate poisoned individuals, we have attempted to elucidate some of its pharmacological actions.

METHODS

Initial experiments were carried out in cats anesthetized with chloralose and prepared by inserting a cannula connected to a Harvard pressure transducer into the carotid or femoral artery. Respiration was recorded by means of an impedance pneumograph and heart rate by a cardiotachometer. From the literature, two doses of HS-6 were selected for study to examine some of the cardiovascular effects of HS-6 (Schenk et al., 1976; Wolthuis et al., 1976). HS-6 or other compounds were injected via the femoral vein and the changes in some cardiovascular and respiratory parameters recorded.

Dose

In those studies where HS-6 was used for studying its gross effects on physiological parameters, the doses are given in mg/kg i.v. In studies comparing the potency of HS-6 with other ganglionic blocking agents, the dose is given as µmoles/kg.

Obtaining Blood Samples

Following the i.v. injection of 30 mg/kg or 100 mg/kg HS-6, aliquots of blood (1 ml) were collected from a cannula inserted in the femoral artery. The HS-6 was injected over a 30-second period and samples were collected at 1, 5, 10 and 20 minutes. In other experiments, blood was drawn following the use of HS-6 at concentrations sufficient to ameliorate the pressor responses to dimethylphenylpiperazinium iodide (DMPP).

Blood Levels of HS-6

The blood levels of HS-6 were determined in a manner similar to that described by Wolthuis et al. (1976) with the following modifications introduced by Clement (1977): 1.0 ml of blood was mixed with 1.0 ml of 10% TCA, vortexed and centrifuged at 0°C. One ml of the supernatant was
transferred to 3.4 ml Tris buffer pH 8.8, mixed and read immediately in a Beckman DU spectrophotometer at 356 nm.

Evidence of Ganglionic Blockade

(a) Carotid Occlusion

Animals were prepared as outlined in the previous sections and a ligature was looped around the carotid artery. Control blood pressures were recorded when the artery was occluded with the ligature. Two minutes following the injection of HS-6, 30 mg/kg, the procedure was repeated and the blood pressure response to carotid occlusion was repeated and compared with control values.

(b) Experiments with Nicotine

A group of cats were treated with a dose of nicotine (35 µg/kg) which caused a reproducible pressor response. After collecting control data on each animal, various doses of hexamethonium or HS-6 were chosen which inhibited the response to nicotine in a dose-related manner. One of the two drugs was then injected intravenously in appropriate doses and 15-20 seconds later the standard dose of nicotine was injected and the pressor response again recorded. The dose of hexamethonium or HS-6 which reduced the nicotine response by 50 per cent was calculated by linear regression analysis and the doses compared at the ED₅₀.

(c) Experiments with Dimethylphenylpiperazinium Iodide (DMPP)

The same protocol as outlined above for nicotine was followed using the ganglion stimulant DMPP alone and with HS-6. The doses of HS-6 which reduced the pressor response to DMPP were recorded and compared with the values obtained with DMPP alone.

(d) Experiments with Adrenaline

Another group of cats were prepared and were injected with adrenaline at a dose which resulted in a consistent pressor response. As in the experiments with nicotine and DMPP, HS-6 was given 15-20 seconds prior to a dose of adrenaline, and the change in pressor response recorded.
(e) Vagus Stimulation

Cats were anesthetized with chloralose and prepared for physiological recording as outlined previously. One vagus nerve was isolated and stimulated with a train of impulses of 1.3 to 1.6 volts and the depressor response recorded. HS-6 was injected i.v. and 1½ minutes later the vagus stimulation was initiated and the depressor response following HS-6 was compared with control values.

(f) Superior Cervical Ganglion of the Cat

This preparation was basically that described by Paton and Perry (1953) and Trendelenburg and Haeusler (1975). Cats were anesthetized with chloralose intraperitoneally. The common, external and internal carotid arteries and the lingual artery on one side were exposed. The lingual artery was cannulated, the internal carotid tied off and the external carotid artery fitted with a clamp which could be added and removed. The sympathetic trunk was isolated and fitted with bipolar platinum electrodes. A suture was tied through the nictitating membrane and tied to a Harvard heart smooth muscle transducer and movements recorded on a Rikadenki linear recorder.

Various doses of HS-6, hexamethonium or mecamylamine were made up in saline and injected into the lingual artery in a volume of 0.1 ml.

Control contractions of the membrane were obtained by stimulating the pre-ganglionic nerve with square waves of 5 milliseconds duration at a strength of 3 to 5 volts. The contraction of the nictitating membrane was recorded. In some animals pupil dilation was also measured. Various doses of the test compounds were then injected and the relaxation of the membrane and change in pupil diameter recorded.

Blood pressure was recorded by means of a pressure transducer tied into the femoral artery. Heart rate was recorded by means of a
Harvard cardiotachometer and respiration by a Harvard impedance pneumograph.

The results obtained following the use of ganglion blocking drugs were presented as inhibition of contraction of the membrane as per cent of control. Least squares regression lines were calculated and the compounds compared at the ED$_{50}$ for potency.

RESULTS

The decrease in mean blood pressure following 30 mg/kg HS-6 given over a 30 second interval is depicted in Figure 2. In these experiments the blood pressure began to fall within seconds and reached 40-50 mm/Hg below control values at from one to two minutes post-injection, followed more slowly by a rise toward control levels. The response of the heart rate was somewhat variable but did not change significantly in this group of animals. The blood level of HS-6 is also shown in Figure 2. HS-6 concentrations reached a maximum level (avg. 242 ± 44 μg/ml) after 1 minute and then began to fall toward normal values relatively quickly during the first 5 minutes (127 ± 23 μg/ml) and then decreased more slowly.

Larger doses of HS-6 (100 mg/kg) were given i.v. to four cats resulting in a similar but longer lasting effect on the blood pressure. During the period following these high doses, 3 of the 4 cats stopped breathing and had to be maintained on artificial ventilation for a time. Blood samples were drawn and analysed when cessation of respiration occurred and the concentration of HS-6 in the blood was found to average 864 μg/ml. Since this dose was obviously toxic to the animals, no further experiments were done at this dose level.

Figure 3 illustrates the results obtained from various procedures used to examine further the hypotensive effect produced by HS-6. Thirty mg/kg HS-6 drastically reduced the blood pressure increase produced by carotid occlusion indicating that it interfered with this reflex pathway. Also shown on the figure is the decreased response of the cats'
FIGURE 2: The rapid fall in blood pressure with the concomitant increase in blood level of HS-6 in the first two minutes following 30 mg/kg i.v. injection of the drug is shown in Figure 2. The large decrease in blood pressure is of short duration and, as it returns to normal, the blood level of HS-6 also falls but more slowly than over the first five minutes.
FIGURE 3: The blood pressure response to carotid occlusion, and the partial blockage of this response two minutes following 30 mg/kg HS-6, is shown in the first panel. A standard dose of either nicotine or DMPP elicited a marked pressor effect which was increasingly blocked by increasing doses of HS-6 given 20 seconds previously. Adrenaline also caused a marked increase in blood pressure, however HS-6 did not attenuate the response. Stimulation of the vagus nerve produced a marked decrease in blood pressure which was partially blocked by HS-6 (30 μ moles/kg).
blood pressure to two drugs which cause a pressor effect via ganglionic stimulation (nicotine and DMPP) and one which does not (adrenaline). HS-6 at the two higher concentrations used in this study potentiated the increase in blood pressure elicited by adrenaline. HS-6, 30 μmoles/kg (10.68 mg/kg), also significantly reduced the hypotensive effect of vagus stimulation.

The relative potency of hexamethonium and HS-6 (84/1) against the pressor response to nicotine is illustrated in Table I. Table I shows that relatively small doses of HS-6, i.e. doses that would probably be exceeded during therapy, progressively blocked the nicotine induced pressor response.

During these experiments blood samples were taken immediately after a dose of HS-6 which attenuated the response to nicotine or DMPP. HS-6 (20 μmoles/kg) which blocked approximately 50% of the nicotine or DMPP induced pressor effect produced blood levels in the range of only 15-25 μg/ml. Thirty μmoles HS-6 produced blood levels of 40-50 μg/ml while totally blocking the pressor response, indicating clearly that significant effects of HS-6 on sympathetic ganglia occur at relatively low blood concentrations in relation to the levels reached following 30 mg/kg.

The contraction of the nictitating membrane following various drug treatments is shown in the next two Figures (4 and 5). Figure 4 shows the normal responses of the membrane to stimulation of the nerve, 'A', followed by the blocking effect produced by hexamethonium 0.5 μmoles,'B'; another control contraction at 'C' is shown followed by a larger dose of hexamethonium at 'D' sufficient to totally abolish the contraction, while at 'E' a control injection of saline is shown which had no effect. Figure 5 illustrates the control contraction of the membrane at 'A' and 'C' with the partial blocking effect of 5 μmoles HS-6 shown at 'B'. The lack of effect of an injection of saline to the blood supply of the ganglion is illustrated in panel 'D' while the increasing blockade of the contraction of the membrane by 10 μmoles HS-6 is shown in panel 'E'.
FIGURE 4: Panel B illustrates the partial blockade of the contraction as compared with panels A, C, and D which show control contractions before and after hexamethonium. Panel D illustrates the response to a larger dose of hexamethonium and E the response to an injection of saline.
Figure 5: Panel B illustrates the partial blockade of the contraction of the isolated smooth muscle preparation in the presence of the higher concentration of the drug, and panel C shows the normal response. Panel F shows the greater proportion of the response. Panel A shows the smooth muscle preparation directly into the blood supplying the smooth muscle.
Panel 'F' shows that HS-6 20 μmoles had no effect on the preparation when injected in the blood supply of the smooth muscle of the nictitating membrane.

Table II shows the effect of increasing doses of HS-6, hexamethonium and mecamylamine on the contraction of the nictitating membrane. All three compounds progressively decreased the response of the nictitating membrane to pre-ganglionic stimulation. Mecamylamine was found to be the most potent, whereas HS-6 was the least potent. In this particular group of animals, hexamethonium was 111 times as potent as HS-6. All three drugs were also noted to progressively block the pupil dilation which accompanied the contraction of the membrane.

DISCUSSION

The results of this study showed clearly the decrease in blood pressure resulting from administration of HS-6 in doses likely to have some value in the therapy of soman poisoning. Wolthuis et al. (1976) used atropine plus 100 mg/kg i.v. HS-6 in the treatment of rats against soman, while Schenk et al. (1976) used a regimen containing 100 mg/kg i.m. HS-6 in dogs. Studies reported here utilizing doses of 30 mg/kg significantly reduced blood pressure, while smaller amounts (in the range of 10 mg/kg) also consistently reduced blood pressure although the decrease was not as dramatic. Other compounds with oxime groups have been shown to demonstrate a variable effect on blood pressure. Brown et al. (1957) reported a pressor effect following various concentrations of 2-PAM. Calesnick et al. (1967) reported that in man 2-PAM i.m. or i.v. up to 45 mg/kg caused a long-lasting increase in blood pressure. Similar results were obtained with its methyl methane sulfonate derivative, P2S. TMB-4, a bisquaternary oxime with a structure similar to HS-6, caused a decrease in blood pressure in animals and man (Calesnick et al., 1967; Lindgren and Sundwall, 1960; and McNamara, 1976). On the other hand, Sidell and Groff (1970) showed a pressor effect and tachycardia in man following toxogonin, an analogue of both TMB-4 and HS-6, while Smirnova et al. (1975) claimed that toxogonin had no cardiovascular effects at
therapeutic doses. It seems certain therefore that the oxime group itself plays little role in the cardiovascular effects of these various cholinesterase reactivators. It is also worth noting that oximes with similar structures appear to produce quite opposite cardiovascular effects.

The blood level of HS-6 following 30 mg/kg i.v. initially rose to high levels (avg. over 240 μg/ml) but quickly fell towards control values. At i.v. doses of 100 mg/kg (the same used in rats by Wolthuis et al., (1976)) the toxicity became evident, as 3 out of 4 cats given this amount ceased breathing with a concomitant blood level of over 800 μg/ml HS-6. Wolthuis et al. (1976) reported that animals which had received HS-6 before receiving soman had much higher blood levels of the oxime than did animals treated with an equal dose of HS-6 alone, and the toxicity seen in these animals was partly attributed to the oxime. The results obtained from this series of cats support the contention that high blood levels of HS-6 cause respiratory paralysis or sensitize respiratory centres to depression by other agents.

Various pharmacological manipulations in the experimental animals were carried out before and after HS-6 administration. Some of these procedures involved autonomic ganglia in the production of a physiological response and some did not. HS-6 progressively blocked the physiological responses which depend on normal ganglionic transmission. The blood pressure response elicited by carotid occlusions was largely obliterated by prior treatment of the animal with HS-6. In addition, doses of HS-6 (3.59-10.77 mg/kg) much less than those so far shown effective against soman progressively, and at the highest dose almost completely, abolished the pressor response to both nicotine and DMPP. Schenk et al. (1976) have shown that blood levels of HS-6 greater than 100 μg/ml are reached in animals receiving 50 mg/kg HS-6 i.m., while in these studies blood levels of HS-6 no higher than 20 μg/ml were present at the same time that ganglionic responses were greatly diminished. HS-6 (30 μmoles/kg) also partially blocked the depressor response elicited by vagal stimulation but did not inhibit the pressor response resulting from adrenaline, a response not mediated by ganglia. HS-6 also attenuated the res-
piratory effects of nicotine in particular.

HS-6 was compared with hexamethonium and mecamylamine for its blocking properties in the cat superior cervical ganglion preparation. Injection of small amounts of HS-6 into the blood supply of the ganglion blocked the effect of pre-ganglionic stimulation in a dose-related manner as did hexamethonium and mecamylamine. All three drugs also blocked the pupil dilation resulting from pre-ganglionic stimulation. The possibility that HS-6 could act directly on the smooth muscle of the nictitating membrane was ruled out by injecting it directly into the blood supply to the muscle (Trendelenburg and Haeusler, 1975). This procedure failed to alter the response of the membrane to stimulation.

The results of these studies clearly show the ganglion blocking properties of HS-6 in concentrations lower than those likely to be used therapeutically in different species. Other oximes of similar structure in current therapeutic use may also have ganglion blocking activity although some disagreement on this subject exists. TMB-4 [1,1-Trimethylene bis (4-formylypyridinium bromide)-dioxide], a structural analogue of HS-6, failed to block the superior cervical ganglion preparation of the cat according to Lindgren and Sundwall (1960), whereas Willems (1977) showed that both TMB-4 and toxogonin progressively blocked the superior cervical ganglion but only in doses too high to be a factor in their therapeutic use.

The above discussion indicates that HS-6 has ganglion blocking properties in therapeutic doses while its congeners TMB-4 and toxogonin, if they block ganglia, do so at doses not likely to be reached during therapy. Other preliminary experiments done in this laboratory indicate that MM-6, [2-hydroxyiminomethyl]-pyridinium-(1) methyl]-[3(2-hydroxyethyl)-pyridinium-(1) methyl] ether dichloride, a congener of TMB-4, toxogonin and HS-6, also has ganglion blocking activity which appears to be slightly more potent than that of HS-6.

Although different theories have been advanced to explain the mechanism of the beneficial effects of HS-6 (or MM-6) against soman poisoning, one factor of pharmacological importance is their ability to interfere with autonomic function during intoxication.
REFERENCES


Calesnick, B., Christensen, J.A. and Richter, M. "Human Toxicity of Various Oximes. 2-Pyridine Aldoxime Methyl Chloride, Its Methane Sulphonate Salt and 1,1' Trimethylene-bis-(4-Formyl Pyridinium Chloride)". Arch. Environ. Health 15:599-608 (1967)


McNamara, B.P. "Oximes as Antidotes in Poisoning by Anticholinesterase Compounds". Edgewood Arsenal. EB-SP-76004 (1976)


Willems, J.L. Heymans Institute of Pharmacology. Ghent, Belgium. Personal communication. (1977)

TABLE I

THE EFFECT OF HS-6 AND HEXAMETHONIUM ON THE PRESSOR EFFECTS OF NICOTINE

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>mean Blood Pressure</th>
<th>No. of</th>
<th>ED$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmoles/kg</td>
<td>increase mm/Hg ± sem</td>
<td>Responses</td>
<td>µmoles/kg</td>
</tr>
<tr>
<td>Nicotine</td>
<td>9.9</td>
<td>62.1 ± 3.24</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Nicotine + HS-6</td>
<td>9.9</td>
<td>36 ± 4.7*</td>
<td>5</td>
<td>15.2</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>21 ± 7.8*</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>2 ± 1.0*</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Nicotine + Hexamethonium</td>
<td>9.9</td>
<td>44 ± 2.3*</td>
<td>6</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>25 ± 6.6*</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>1 ± 1 *</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Ratio HS-6/Hexamethonium 1/84

Cats given nicotine 20 seconds following the administration of doses of either HS-6 or hexamethonium showed a progressive attenuation of the pressor response with increasing doses of the two compounds.

* denotes responses which are statistically different from nicotine alone p<0.05

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**TABLE II**

THE EFFECT OF HS-6, HEXAMETHONIUM AND MECAMYLANE
ON THE CAT - NICTITATING MEMBRANE PREPARATION

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of Animals</th>
<th>Dose μmoles</th>
<th>% Inhibition of Contraction ± sem</th>
<th>ED$_{50}$ μmoles</th>
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<tr>
<td>HS-6</td>
<td>4</td>
<td>1.0</td>
<td>13 ± 2.1*</td>
<td>5.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>50 ± 11.4*</td>
<td></td>
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<td></td>
<td></td>
<td>10.0</td>
<td>80 ± 11.8*</td>
<td></td>
</tr>
<tr>
<td>HEX</td>
<td>4</td>
<td>0.01</td>
<td>15 ± 4.2*</td>
<td>0.051</td>
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<td></td>
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<td>0.05</td>
<td>50 ± 10.2*</td>
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<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>95 ± 7.0*</td>
<td></td>
</tr>
<tr>
<td>Mecamylamine</td>
<td>4</td>
<td>0.02</td>
<td>34 ± 2.1*</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03</td>
<td>66 ± 6.5*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.04</td>
<td>100 *</td>
<td></td>
</tr>
</tbody>
</table>

Ratio HS-6/Hexamethonium 1/111 at ED$_{50}$
Ratio HS-6/Mecamylamine 1/217 at ED$_{50}$

HS-6, hexamethonium and mecamylamine injected into the blood supply of the cats superior cervical ganglion progressively blocked the contraction of the nictitating membrane in response to pre-ganglionic stimulation.

* denotes a statistically significant reduction of membrane contraction  
$\ p<0.05$
The ganglionic blocking properties of the cholinesterase reactivator HS-6 (U)

Following i.v. administration of 30 mg/kg of the cholinesterase reactivator HS-6, blood pressure fell (up to 50 mm/Hg) and maximal blood levels of HS-6 reached 242 µg/ml. HS-6 attenuated the pressor response resulting from carotid occlusion and the depressor effect of vagal stimulation. Doses of HS-6 below those used therapeutically against soman in different animal species (3.59-10.77 mg/kg) progressively blocked the ganglion stimulating effects of nicotine and dimethylphenylpiperazinium but not those following adrenaline, a pattern similar to that produced by hexamethonium but only 1/84 as potent. HS-6, like hexamethonium and mecamylamine, progressively blocked the contraction of the nictitating membrane of the cat resulting from pre-ganglionic stimulation.

The results indicate that HS-6 possesses ganglion blocking properties at doses likely to be used therapeutically in soman poisoning. The ganglion blocking properties of the drug may be a factor in the beneficial effects of HS-6.
KEY WORDS

HS-6
Cholinesterase Reactivator
Hypotension
Ganglion Blocking Agents
Toxicity

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