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THE EFFECT OF ESERINE AND NEOSTIGMINE ON THE BLOOD PRESSURE OF CONSCIOUS RATS

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Small amounts of eserine salicylate (10 to 20 μg.) regularly caused a rise of blood pressure in non-anaesthetized rats. Neostigmine methylsulphate in doses from 1 to 10 μg./animal usually caused a fall of blood pressure, or no change was observed; only in a few experiments was a rise of blood pressure noted. The pressor effect of eserine was abolished by atropine and reduced or abolished by yohimbine and phentolamine. Adrenalectomy did not change the response to eserine. The present experiments do not contradict earlier statements that the pressor effect of eserine was due to the discharge of impulses from a centre or centres in the central nervous system.

Several workers have observed that eserine raises the blood pressure of rats anaesthetized with urethane (Dirnhuber and Cullumbine, 1955; Varagić, 1955; Hornykiewicz and Kobinger, 1956). This effect of eserine was attributed to the discharge of impulses from a centre or centres in the brain at points not lower than the medulla.

It is generally known that the effect of some substances on the blood pressure of the same animal may depend on the anaesthetic agent and on the manner of preparation. We therefore decided to test the effect of eserine in non-anaesthetized rats. The influence of hexamethonium, of adrenalectomy, of atropine, and of antisympathomimetic agents on this effect was also studied.

METHODS

Male and female albino rats of 125 to 200 g. were used. For the indirect measurement of the blood pressure in the rat, a tail plethysmograph, described in detail by Van Proosdij-Hartzema (1954), was used. This method permits repeated blood-pressure measurements over a long period. Its principle is to exclude the vessels of the tail from the circulation by means of an external pressure exerted on the proximal part of the tail by an occluding cuff. This pressure is gradually released, and the value at which the inflow of blood starts again is read on a mercury manometer. In most experiments, the temperature of the fluid in the plethysmograph was kept between 41 and 44° C.

The drugs tested were injected into the tail vein in a volume of 0.3 ml.; only cocaine hydrochloride was injected intraperitoneally.

RESULTS

Action of Various Anticholinesterase Agents.—The effect of eserine salicylate on the blood pressure of non-anaesthetized rats is shown in Table I. In this series of experiments 10 μg. eserine salicylate was injected into each animal (60 to 77 μg./kg.). This dose caused a rise of blood pressure ranging from 24 to 95 mm. and lasting 20 to 57 min. The rise of blood pressure reached its maximum 4 to 16 min. after the injection of eserine. The increase in blood pressure was 12 to 158% of the initial level.

In another group of three rats, 20 μg. eserine salicylate (101 to 153 μg./kg.) caused a rise in blood pressure ranging from 56 to 76 mm. The duration of the effect was 76 to 99 min.

TABLE I

THE EFFECT OF INTRAVENOUS ESERINE SALICYLATE (10 μG.) ON THE BLOOD PRESSURE OF NON-ANAESTHETIZED RATS

No. of Expt.	Initial Blood Pressure (mm. Hg)	Maximum b.p. after Eserine (mm. Hg)	Maximum Reached after (min.)	Duration of Effect (min.)	% Increase over Initial Level
1	58	96	11	57	65
2	66	112	12	41	69
3	60	155	7	45	158
4	64	128	15	30	100
5	85	131	16	—	54
6	49	100	12	24	104
7	94	118	4	29	12
8	60	106	16	55	76
9	66	123	7	54	86
10	123	147	8	29	19
11	72	110	5	57	53
12	75	118	11	20	57
Mean	82	120	10	40	71

The effect of neostigmine methylsulphate was tested in a group of 14 rats. In nine of them neostigmine in doses of 2 to 5 μg. (13 to 33 μg./kg.) caused a fall of blood pressure ; in three the same dose caused no change of blood pressure ; and in two rises of 17 and 25 mm. were observed.

Action of Hexamethonium.—In rats anaesthetized with urethane, large amounts of hexamethonium (C_6) (128 to 167 mg./kg.), given in one or two injections, abolished the pressor action of eserine in doses of 30 to 90 μg./kg. (Varagić, 1955). Dirnhuber and Cullumbine (1955) used even larger doses of C_6 (200 to 400 mg./kg.) to abolish the pressor effect of another anticholinesterase agent, sarin (isopropyl methylphosphonofluoridate).

In the present experiments, C_6 itself in a dose of 30 mg./kg. caused a fall in blood pressure ranging from 10 to 69 mm. and lasting 4 to 15 min., except for one out of nine experiments when the fall lasted more than 30 min. Similar effects were observed after 40 mg./kg. C_6. A dose of 50 mg./kg. C_6 caused either no change in blood pressure or a rise ranging from 20 to 30 mm. This dose of C_6 killed four out of nine animals. Van Proosdij-Hartzema and De Jongh (1955) found that 5 and 20 mg./kg. C_6, given orally, hardly influenced the blood pressure, whereas 40 mg./kg. caused a slight rise.

The pressor effect of eserine in non-anaesthetized rats was not greatly affected by C_6 in doses of 30 to 50 mg./kg. Both potentiation and reduction of the effect of eserine were observed after these doses of C_6. In Fig. 1 the dose of 10 μg. eserine caused a rise of blood pressure of 25 mm. Before

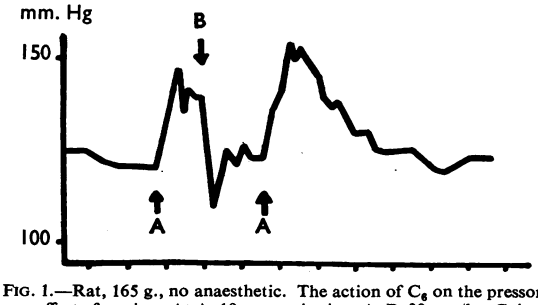

FIG. 1.—Rat, 165 g., no anaesthetic. The action of C_6 on the pressor effect of eserine. At A, 10 μg. eserine i.v. At B, 30 mg./kg. C_6 i.v. Note the potentiation of the pressor effect of eserine by C_6. Time in 10 min. intervals.

the effect of eserine had ceased, 30 mg./kg. C_6 was injected and it caused an abrupt fall in blood pressure. The injection of eserine was then repeated and it caused a rise of blood pressure of 30 mm. The opposite effect was obtained in another experiment. In this rat 10 μg. eserine caused a rise of blood pressure of 59 mm. When the pressure had returned to the initial level, 50 mg./kg. C_6 was injected and no change in blood pressure was observed. The repeated injection of 10 μg. eserine caused a rise in blood pressure of only 24 mm.

On the other hand, C_6 reduced the pressor effect of eserine if injected immediately after this drug. A typical experiment is shown in Fig. 2. In this rat 20 μg. eserine (117 μg./kg.) caused a rise of blood pressure of 61 mm. When the effect of eserine had reached a maximum, 30 mg./kg. of C_6 was injected. It caused a fall of blood pressure, but it did not completely abolish the effect of eserine, which was still evident 55 min. after the injection of C_6.

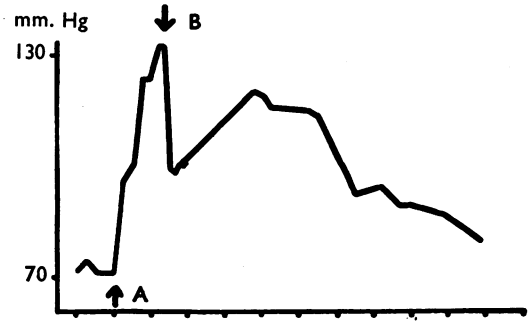

FIG. 2.—Rat, 170 g., no anaesthetic: The action of C_6 on the pressor effect of eserine. At A, 20 μg. eserine i.v. At B, 30 mg./kg. C_6 i.v. Time in 10 min. intervals.

Action of Eserine in Adrenalectomized Rats.—It has been known for a long time that eserine can produce a 10- to 15-fold increase in the output of adrenaline from the adrenals of the cat (Stewart and Rogoff, 1921). We thought that the pressor effect of eserine in the rat might be due to the liberation of adrenaline from the adrenals. A series of rats was accordingly adrenalectomized and, 9 to 18 days later, the effect of eserine on their blood pressures was tested. As will be seen from Table II, there was no significant difference between the duration of the effect in normal and in adrenalectomized rats.

The effect of C_6 in adrenalectomized rats was very similar to that observed in normal rats. Thus, in a group of three rats in which eserine was tested 8, 9 and 18 days after adrenalectomy, C_6 in a dose of 30 mg./kg. did not change the pressor effect of 10 μg. eserine. In another rat, 12 days after adrenalectomy, even 60 mg./kg. C_6, given in two injections, did not change the response to 10 μg. eserine. Only in one out of five experiments did

TABLE II

THE EFFECT OF INTRAVENOUS ESERINE SALICYLATE (10 μG.) ON THE BLOOD PRESSURE OF NON-ANAESTHETIZED ADRENALECTOMIZED RATS

No. of Expt.	Initial Blood Pressure (mm. Hg)	Maximum b.p. after Eserine (mm. Hg)	Maximum Reached after (min.)	Duration of Effect (min.)	% Increase over Initial Level
1	64	98	12	30	53
2	86	132	3	20	53
3	88	123	4	14	39
4	72	118	4	27	63
5	87	128	4	37	47
6	98	122	11	31	24
7	40	95	5	49	136
Mean	76	115	6	30	59

C_6 (30 mg./kg.) abolish the pressor effect of eserine 20 μg. (140 μg./kg.).

Abolition of Response by Atropine and Phentolamine.—Atropine sulphate in doses 2 to 7 mg./kg. completely abolished the pressor effect of eserine.

Yohimbine in doses of 5 to 10 mg./kg. distinctly reduced the effect of eserine, whereas phentolamine (Regitin) in doses from 7.5 to 30 mg./kg. reduced or even abolished the response to eserine, as shown in Fig. 3. Both phentolamine and yohimbine alone caused a fall of blood pressure.

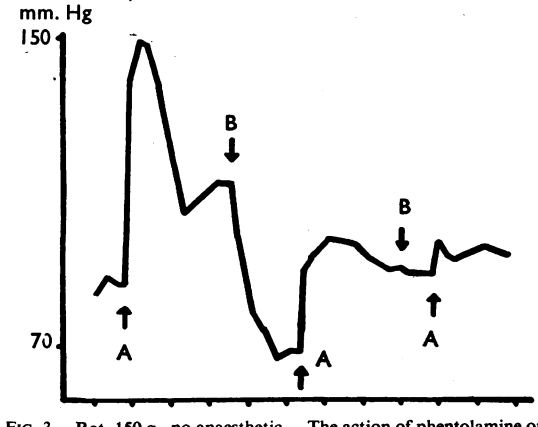

FIG. 3.—Rat, 150 g., no anaesthetic. The action of phentolamine on the pressor effect of eserine. At A, 20 μg. eserine i.v. At B, 10 mg./kg. phentolamine i.v. Note abolition of the effect of eserine by phentolamine. Time in 10 min. intervals.

Action of Cocaine.—It has been shown on rats under urethane that the pressor effect of eserine was increased after cocaine (Varagić, 1955). In non-anaesthetized rats measurement of the blood pressure by the method used in these investigations was impossible after intraperitoneal injection of cocaine (5 to 7 mg./kg.)—most probably because of constriction of the blood vessels in the tail.

DISCUSSION

The results obtained in the present experiments are in accord with the findings in the rat under urethane. In doses of 10 to 20 μg. (60 to 153 μg./kg.), eserine regularly caused a rise of blood pressure in non-anaesthetized rats. The previous studies on rats under urethane showed that the pressor effect of eserine was central in origin (Dirnhuber and Cullumbine, 1955 ; Varagić, 1955 ; Hornykiewicz and Kobinger, 1956), and that it was exerted at points not lower than the medulla. The present experiments do not contradict this view.

The pressor effect of eserine is not common to all anticholinesterase agents because neostigmine methylsulphate did not show this effect. Similar differences between eserine and neostigmine were observed by Eccles, Fatt, and Koketsu (1954) and Eccles, Eccles, and Fatt (1956) during pharmacological investigations on a central synapse at which acetylcholine was a transmitter. Thus, eserine greatly prolonged the duration of the discharge of impulses which an antidromic volley evoked from a Renshaw cell. In contrast to eserine, neostigmine was much less effective, even in a dose as large as 2 mg./kg. One possible explanation for the relative ineffectiveness that is usually observed for neostigmine given by intravenous injection is that a barrier to the diffusion of this quaternary amine surrounds the blood vessels of the central nervous system, whereas eserine, being a tertiary compound, can reach the central nervous system more readily. However, neostigmine would appear to be at least as effective as eserine in prolonging the discharge of impulses when applied to the environment of the Renshaw cell (Eccles *et al.*, 1956), or in causing a rise in blood pressure when injected intracisternally (Hornykiewicz and Kobinger, 1956).

The effect of eserine was not abolished by C_6 even in doses from 30 to 50 mg./kg., the last dose being close to the LD50 for our animals. In a rat under urethane, 128 to 160 mg./kg. C_6 had to be given in one or two injections in order to abolish the pressor action of eserine. It is difficult to explain why non-anaesthetized rats were more sensitive to C_6 than were rats under urethane. In non-anaesthetized rats the doses necessary to abolish the pressor effect of eserine could not be reached without killing the animals.

Liberation of adrenaline and noradrenaline from the adrenals does not seem to be responsible for the blood-pressure rise after eserine. The effect of eserine in the adrenalectomized rat was qualitatively the same as in the controls. It is

difficult to say whether the effect of eserine was easier to abolish by C_6 in the adrenalectomized than in the control rats. The fact remains that the only rat in which the effect of eserine was abolished after 30 mg./kg. C_6 was the adrenalectomized one. This might indicate that larger amounts of C_6 are necessary to block the adrenals than to block the sympathetic ganglia. On the other hand, the difficulty of blocking the effect of eserine by C_6 in normal rats might be due to potentiation by C_6 of the effect of small amounts of adrenaline and noradrenaline liberated from the adrenals.

Atropine sulphate in doses from 2 to 7 mg./kg. abolished the effect of eserine. The adrenaline antagonists yohimbine and phentolamine distinctly reduced or even abolished the effect of eserine. The effect of atropine does not seem to result from its ganglionic blocking action, because Cahen and Tvede (1953) found that in cats 6 to 15 mg./kg. atropine was necessary to block the ganglia controlling the blood pressure. Most probably, atropine acts centrally. Reduction or abolition of the effect of eserine by adrenaline antagonists would mean that the pressor effect of eserine was mediated by sympathetic impulses coming from the higher centres to the walls of the blood vessels.

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