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THE ACTION OF ESERINE ON THE BLOOD PRESSURE OF THE RAT

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Recently α-cocaine was prepared in this department under the direction of Dr. H. R. Ing by Dr. R. Foster. In the course of pharmacological experiments on the action of α-cocaine, which will be described in another paper, it was decided to test its action on the pressor effect of adrenaline when eserine had been given.

The experiments were to be performed on cats, but as the supply of cats was short the observations were made in rats. It was then found that eserine had a pressor action in the rat, and the experiments now described were undertaken to analyse this action. During the course of the work a paper appeared by Dirnhuber and Cullumbine (1955) which also describes the pressor effect of certain anticholinesterase agents in the rat. Since my observations confirm many of their findings, attention will be given mainly to new points or points of difference from them.

METHODS

In general, the method for recording the rat's blood pressure described by Crawford and Outschoorn (1951) has been followed. Rats (200–400 g.) of both sexes were used, and anaesthetized with 0.7 ml. per 100 g. of body weight of 25% urethane solution subcutaneously. To record the blood pressure a cannula was inserted into the carotid artery and connected with a mercury manometer, as described in detail by Condon (1951). A small polythene cannula, 0.5 mm. in diameter, was inserted into the jugular vein, and was used for injecting drugs. Before the experiment was started 1–1.5 mg./100 g. of heparin was injected. All doses of drugs were injected in 0.1 ml. and washed in with the same volume of saline. In most of the experiments artificial respiration was applied. The effect of eserine was the same no matter whether the animal was breathing naturally or not.

The hindlegs of rats were perfused by inserting a cannula into the aorta immediately before the bifurcation. The perfusion fluid was oxygenated Locke solution. In a few experiments 25% "Intradex" in Locke solution was used. The venous outflow was recorded by means of Stephenson's recorder (1948).

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RESULTS

Action of Different Anticholinesterase Agents.—The effect of the intravenous injection of 10 μg. eserine sulphate into a rat of 230 g. weight is shown in Fig. 1C. This amount of eserine, equivalent to 43 μg./kg., caused a rise of pressure equal to 50 mm. Hg which lasted for about 20 min. Such an effect was observed in 60 experiments varying from one rat to another only in duration; the longest duration was 45 min. and the shortest was 6 min. Dirnhuber and Cullumbine observed similar effects with sarin (*iso*propylmethylphosphonofluoridate), dyflos (di*iso*propylphosphorofluoridate), TEPP (tetraethylpyrophosphate) and E.600 (diethyl-*p*-nitrophenylphosphate). With other anticholinesterase agents I failed to observe the pressor action. Thus in Fig. 1A the injection of 25 μg. Nu 683 (the dimethylcarbamate of 2-hydroxy-5-phenylbenzyltrimethylammonium bromide) caused a fall of pressure. Similarly in Fig. 1B the injection of 25 μg. Nu 1250 (*N*-*p*-chlorophenyl-*N*-methylcarbamate of *m*-hydroxyphenyltrimethylammonium bromide) failed to cause a rise of pressure. Finally, in Fig 1D the injection of 10 μg. BW284C51 (the dimethobromide of 1:5-di-(*p*-*N*-allyl-*N*-methylaminophenyl)-pentan-3-one) caused an insignificant rise. Neostigmine (Prostigmin methylsulphate) was also ineffective in causing a rise of pressure which was at all comparable with that produced by eserine. In three experiments the injection of 10 μg. neostigmine caused a fall of blood pressure. In one of these the injection of 30 μg. neostigmine caused a rise of 13 mm. and the injection of 100 μg. caused a rise of 20 mm. In a fourth experiment the injection of 30 μg. caused a rise of 22 mm., but a repetition of the injection caused a fall.

Action of Nicotine.—The pressor effect of eserine was found to be abolished in some rats by nicotine as shown in Fig. 2. At the arrow 10 μg. eserine was injected into a rat of 225 g. and the injection produced the usual rise. At 1, 50 μg.

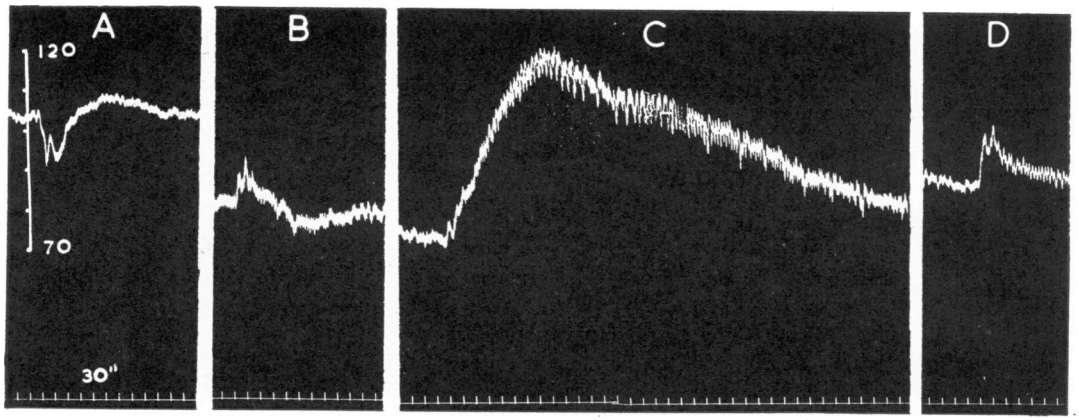

FIG. 1.—Rat, 230 g., under urethane. The action of various anticholinesterases on arterial blood pressure. A, 25 μg. Nu 683 i.v. B, 25 μg. Nu 1250 i.v. C, 10 μg. eserine i.v. D, 10 μg. BW284C51 i.v. Note that only eserine caused an appreciable rise of blood pressure.

nicotine acid tartrate was injected, causing a transient rise of pressure; at 2, 100 μg. nicotine was injected; at 3 and 4, 1 mg. nicotine was injected. The injection of 10 μg. eserine then failed to cause any rise when given twice, and 30 μg. eserine was also inactive. Thus the total of 9.5 mg./kg. of nicotine abolished the action of eserine. The same result was obtained in two other experiments, the amounts of nicotine injected being equivalent to 6.2 and 11.8 mg./kg. respectively. In three other experiments injections of nicotine made at longer intervals failed to abolish the action of eserine although the total amount was 3.55, 1.3, and 3.55 mg./kg. respectively. However, when relatively small amounts of hexamethonium were given in addition, namely, 1.73, 7.6, and 3.8 mg./kg., the pressor effect of eserine was abolished.

Action of Hexamethonium.—Dirnhuber and Cullumbine stated that sarin caused a rise of pressure in the presence of amounts of hexamethonium equivalent to 10–20 mg./kg.; after large doses of hexamethonium from 200–400 mg./kg., sarin then had no effect. In my experiments with eserine large amounts of hexamethonium (128, 142, and 167 mg./kg. in three experiments) given in one or two injections abolished the pressor action of amounts of eserine in the range of 30–90 μg./kg.; but amounts of eserine such as 1.5 mg./kg. were still active.

The effect of hexamethonium in abolishing the pressor effect of eserine was much greater after adrenalectomy. For example, in two adrenalectomized rats 1.43 and 5.0 mg./kg. were sufficient to abolish the pressor effect. In two other rats 3.8 and 4.2 mg./kg. hexamethonium did not reduce the action of eserine before adrenalectomy; after adrenalectomy, however, the injection of 2.8 and 4.2 mg./kg. abolished the pressor effect. A similar effect was observed by Dirnhuber and Cullumbine when investigating sarin.

In the experiment shown in Fig. 3 the effect of 30 μg. eserine shown in A was reduced by injection of 0.8 mg. C6 to that shown in B. In the course of the next 70 min. a series of 11 injections of 0.4 mg. C6 were given, and the effect of 30 μg. eserine was increased to the point shown in C. Injections of nicotine were then given up to a total of 9.85 mg. These did not modify the action of eserine as shown in D.

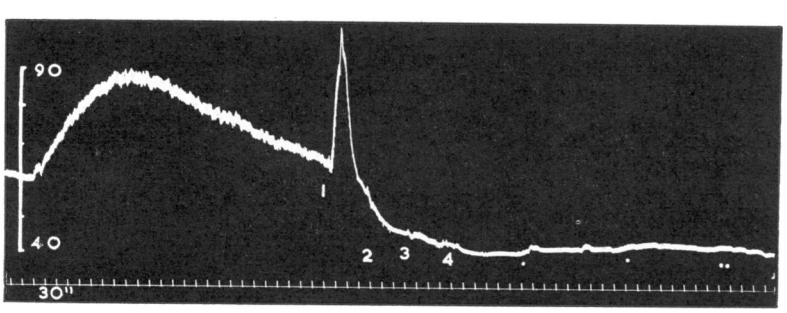

FIG. 2.—Rat, 225 g., under urethane. The action of nicotine on the pressor effect of eserine injected i.v. At one white spot, 10 μg. eserine. At 1, 50 μg. nicotine acid tartrate. At 2, 100 μg. nicotine acid tartrate. At 3 and 4, 1 mg. nicotine acid tartrate. At two white spots, 30 μg. eserine. Note complete abolition of the effect of eserine after nicotine.

Potentiation by Cocaine.—The pressor effect of eserine was increased by cocaine as shown in Fig. 4. In Fig. 4A is shown the rise of pressure produced by 5 μg. eserine; 0.75 mg. cocaine hydrochloride was then injected into the peritoneal cavity. The response

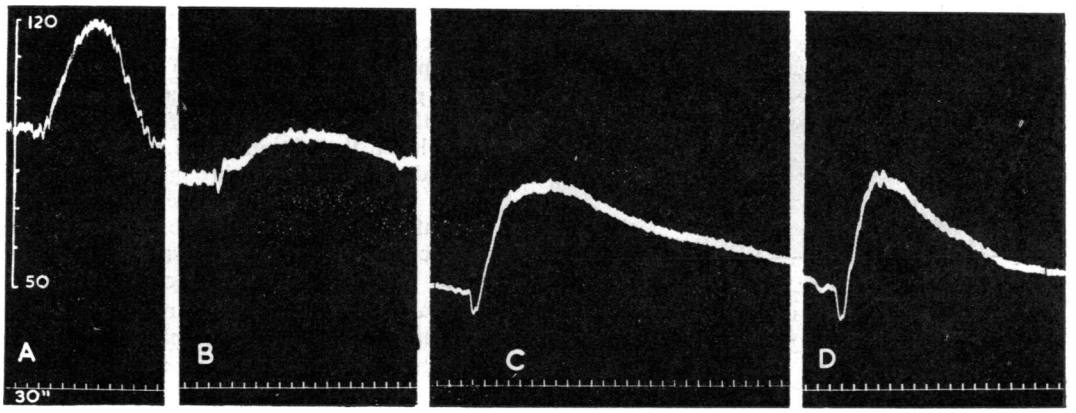

FIG. 3.—Rat, 230 g., under urethane. The action of hexamethonium and nicotine on the pressor effect of eserine. At A, B, C and D, injection of 30 μg. eserine. Between A and B 0.4 mg. hexamethonium was injected twice. Between B and C 0.4 mg. hexamethonium was injected eleven times. Between C and D 9.85 mg. nicotine acid tartrate was injected. Note decrease of the effect of eserine after small doses of hexamethonium, and return of the effect after larger doses. Note also that after hexamethonium nicotine did not alter the effect of eserine.

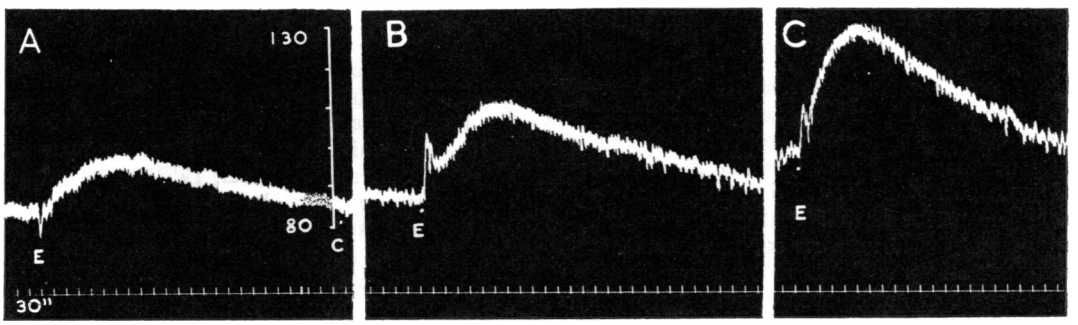

FIG. 4.—Rat, 260 g., under urethane. The action of cocaine hydrochloride on the pressor effect of eserine. At E in A, B and C, 5 μg. eserine was injected i.v. At C in A, 0.75 mg. cocaine hydrochloride intraperitoneally. B was taken 20 min. and C 45 min. after injection of cocaine. Note the potentiation of the pressor effect of eserine by cocaine.

to 5 μg. eserine increased as shown in Fig. 4B, which was 20 min. later, and further increased in Fig. 4C, which was 45 min. later.

Effect of Decerebration.—Eserine (30 μg.) caused a rise of blood pressure in two rats which were decerebrated; the result in one of these is shown in Fig. 5, from which it is evident that the rise was large. In both experiments the natural respiration stopped after the injection, and although respiration was then maintained artificially the blood pressure fell and further injections of eserine were without effect. In spinal rats, eserine had very little effect on the blood pressure, but produced a small rise when doses as large as 0.5 mg. were injected.

Abolition of Response by Dibenamine.—Dirnhuber and Cullumbine found that the pressor effect

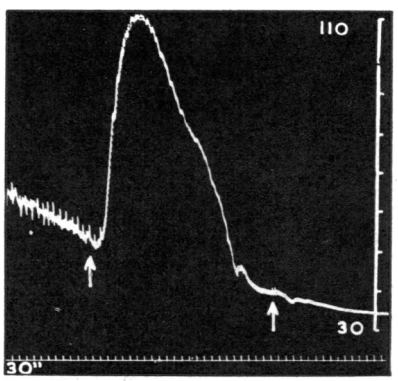

FIG. 5.—Rat, 320 g., decerebrated. At white spot 10 μg. eserine was injected. At arrow 30 μg. eserine was injected. After first dose of 30 μg. eserine the natural respiration stopped and had to be continued artificially. Observe the absence of the effect of eserine at the second injection.

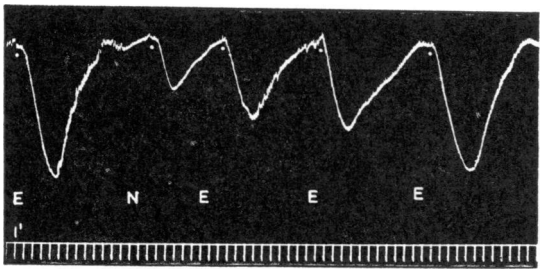

FIG. 6.—Perfusion of rat's hindlegs. The venous outflow recorded by Stephenson's recorder. At E, 1 mg. eserine; at N, 1 mg. nicotine acid tartrate, injected into cannula. Observe the reduction of the effect of eserine after nicotine.

of sarin was reduced to a brief initial spike in the presence of ergotamine or tolazoline. I have found that 1 mg. dibenamine abolished the pressor action of eserine completely. I also observed, in confirmation with Dirnhuber and Cullumbine, that the pressor effect was abolished by atropine, but was not appreciably affected by adrenalectomy or by evisceration. The response was also unaltered by section of the splanchnic nerves.

Peripheral Constrictor Action of Eserine.—To determine whether eserine exerted any part of its pressor effect by a direct action on the vessels, experiments were performed in which the hindlegs of rats were perfused through a cannula tied in the aorta. It was found that when eserine in doses of 1 mg. was injected into the cannula it produced a diminution in venous outflow as shown in Fig. 6. This constrictor action was diminished by an injection of 1 mg. nicotine, and then was restored to its original size by successive injections. The constrictor effect was observed in 11 experiments.

Eserine was not found to affect the rate or amplitude of the isolated rat heart when injected in amounts up to 0.1 mg.

DISCUSSION

The observations described in this paper give general support to the conclusions of Dirnhuber and Cullumbine concerning the mechanism of the pressor effect of sarin in the rat, which they found was also exerted by eserine, dyflos, TEPP, and E.600. While they state that the doses of these substances required to cause a rise of blood pressure were "near-lethal," I have regularly obtained large rises of pressure with 10–30 μg. eserine. The rise was obtained in the decerebrate rat as well as in the rat under urethane. A small rise was also obtained in the spinal rat as Dirnhuber and Cullumbine found with sarin. The main effect on the central nervous system seems, nevertheless, to be exerted at points not lower than the medulla. In agreement with Dirnhuber and Cullumbine the rise of pressure is abolished by atropine, this substance probably acting centrally, and also by hexamethonium and nicotine, from which it may be concluded that the impulses producing the rise of blood pressure pass through the sympathetic ganglia. The rise is augmented by cocaine and absent after the injection of dibenamine, as would be expected if it were due to sympathetic impulses arriving in the walls of the blood vessels.

Liberation of adrenaline and noradrenaline from the adrenal glands appears to play a part in causing the rise of pressure. As long ago as 1921 Stewart and Rogoff observed that after giving eserine to cats the output of adrenaline from the adrenals was increased 10 to 15 times. I found that after the removal of the adrenals much smaller amounts of hexamethonium were sufficient to abolish the rise of blood pressure due to eserine. In the second place, in some experiments, the effect of eserine was depressed as usual by the first injection of hexamethonium, but increased with subsequent injections of hexamethonium as shown in Fig. 3. This change was probably explained by the augmentation of the pressor effect of adrenaline which has been observed by various workers (Slater and Dresel, 1952) to be produced by hexamethonium. It would seem that much larger amounts of hexamethonium are required to block the adrenal glands than to block the sympathetic ganglia, and the effect of the small amount of adrenaline still liberated from the glands due to incomplete block is steadily increased by further injections of hexamethonium.

The augmentation of the pressor effect by cocaine gave further support to the view that the effect was due in part to the release of adrenaline or noradrenaline.

Finally, I observed a constrictor effect of eserine in the hindlegs of the rat perfused with Locke solution through the aorta. This effect was diminished by the injection of nicotine which itself produced constriction; the effect would therefore seem to resemble the constrictor effect of nicotine recently described by Kottegoda (1953) in the perfused rabbit ear. Ambache and Lessin (1955) have also found evidence of a "neuronal" action of eserine in isolated strips of rabbit ileum. Thus, not only in centres in the brain but also at points of the peripheral nervous system, eserine has a stimulant action which is unusually strong in the rat.

Dirnhuber and Cullumbine found that the pressor effect of sarin was shared by eserine, dyflos, TEPP, and E.600. I have observed that the effect

was not obtained with substances containing quaternary nitrogen atoms such as neostigmine, Nu 1250, Nu 683, and BW284C51. This suggests that the former compounds are active because they penetrate the central nervous system more readily.

Summary

1. Small amounts of eserine (10 μg.) caused an appreciable rise of blood pressure in the rat anaesthetized by urethane or decerebrated.

2. In some spinal preparations, also, large amounts (0.5 mg.) caused a rise.

3. This action was not shared by neostigmine or other anticholinesterases containing a quaternary nitrogen atom.

4. The action of eserine was blocked by nicotine and by large amounts of hexamethonium. After adrenalectomy it was blocked by small amounts of hexamethonium.

5. The action was potentiated by cocaine, and blocked by dibenamine.

6. Eserine had some constrictor action in the perfused hindlegs of the rat; this action was depressed by nicotine.

7. The action appeared to be mainly due to the discharge of impulses from a centre or centres in the brain.

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