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FACTORS INFLUENCING THE HYPERTENSIVE EFFECT OF ESERINE IN THE RAT

BY

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Several factors influencing the hypertensive effect of eserine in the rat were investigated. Pretreatment with reserpine regularly depressed or abolished the hypertensive response to eserine. The slow intravenous infusion of either noradrenaline, dihydroxyphenylalanine or 5-hydroxytryptamine only occasionally restored the hypertensive effect of eserine in reserpine-treated rats. Bretylium and choline 2,6-xylyl ether bromide significantly depressed or even abolished the hypertensive effect of eserine. The effect of bretylium was stronger than that of choline 2,6-xylyl ether bromide. Cocaine was found to antagonize the action of bretylium on the response to eserine. In doses which significantly depressed the action of eserine bretylium did not inhibit the hypertension due to excitation of medullary centres induced by clamping the common carotid arteries. Lowering of body temperature abolished the hypertensive effect of eserine. Pretreatment with isopropylisoniazid did not antagonize the inhibitory action of reserpine on the hypertensive response to eserine. It is concluded that the present experiments indicate that the hypertensive effect of eserine in the rat is due to central activation of adrenergic nervous elements. Liberation of noradrenaline (and adrenaline) from the adrenals and from the blood vessels by eserine is an insignificant factor in producing the hypertensive response to eserine.

Eserine raises the blood pressure of the rat anaesthetized with urethane (Varagic, 1955; Dirnhuber & Cullumbine, 1955; Hornykiewicz & Kobinger, 1956) as well as of the conscious rat (Medakovic & Varagic, 1957). This hypertensive effect was attributed to the discharge of impulses from centres in the central nervous system. On the other hand, as long ago as 1921, Stewart and Rogoff observed that in cats the output of adrenaline from the adrenals was increased 10 to 15 times after eserine. In the rat, however, liberation of adrenaline (and noradrenaline) from the adrenals does not seem to play a significant role in the blood pressure rise after eserine because the hypertensive effect of eserine was very much the same in adrenalectomized rats as in the controls (Medakovic & Varagic, 1957).

There is evidence that noradrenaline is present in the walls of the blood vessels (Schmiterlöw, 1948; Burn & Rand, 1958a). This noradrenaline could be liberated by treatment of the animal with reserpine and again restored by an intravenous infusion of noradrenaline (Burn & Rand, 1958a). In reserpine-treated cats some sympathomimetic amines lose their hypertensive activity (Carlsson, Rosengren, Bertler & Nilsson, 1957) which could be restored by intravenous infusion of noradrenaline (Burn & Rand, 1958b). The hypertensive effect of eserine in the reserpine-treated rat is also depressed or abolished (Hornykiewicz & Kobinger, 1956),

but it is not known whether intravenous infusion of noradrenaline or its precursor might restore that effect. Experiments have now been done in order to determine to what extent a peripheral liberation of noradrenaline might contribute to the hypertensive effect of eserine in the rat. By using bretylium, a new type of adrenergic blocking agent, and also by lowering the temperature of the animal, it was possible to obtain some new data which might explain the hypertensive response to eserine.

METHODS

Rats of both sexes (150 to 380 g) were used and anaesthetized with 0.7 ml./100 g body weight of 25% urethane solution injected subcutaneously. To record the blood pressure a cannula was inserted into the carotid artery and connected with a capillary mercury manometer (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 to 1.5 mg/100 g body weight of heparin was injected. All doses of drugs were injected in 0.1 ml. and washed in with the same volume of saline.

Noradrenaline and l-dihydroxyphenylalanine were infused from a microburette through a small polythene cannula in the jugular vein. The rate of infusion ranged from 100 to 1,000 ng/min for noradrenaline, and was 0.25 mg/min for dihydroxyphenylalanine. Reserpine was injected intraperitoneally in a dose of 1 to 2 mg/kg on 2 successive days and on the third day the animal was used for experiment.

Cooling was done by placing pieces of ice over the body of the anaesthetized animal.

The following substances were used: noradrenaline bitartrate, l-dihydroxyphenylalanine, eserine salicylate, cocaine hydrochloride, bretylium tosylate, choline 2,6-xylyl ether bromide, isopropylisoniazid and reserpine ("Serpasil," Ciba).

RESULTS

The effect of noradrenaline on the response to eserine. It was found that previous intravenous infusion of noradrenaline in doses from 1 to 10 μg for 10 min does not significantly alter the blood pressure response to eserine. Only in 3 out of 9 experiments was a slight potentiation of the response to eserine observed. In 3 other experiments the effect of eserine was depressed after infusion of noradrenaline, whereas in the remaining 3 no change was observed.

The effect of reserpine and noradrenaline on the pressor response to eserine. Pretreatment of rats by reserpine in doses from 1 to 2 mg/kg intraperitoneally significantly depressed or abolished the hypertensive response to eserine. These findings confirm those of Hornykiewicz and Kobinger (1956). In reserpine-treated animals the noradrenaline stores in the blood vessels are presumably low. If eserine acted by liberating noradrenaline from the stores it should be possible to restore the response to eserine by slow intravenous infusion of noradrenaline. It was found in the present work that only in 2 out of 11 experiments did infusion of noradrenaline restore the hypertensive effect of eserine, whereas in 9 experiments no change was observed. One of the 2 experiments is shown in Fig. 1, where the usual response to 41.7 μg/kg eserine in the reserpine-treated animal is shown in (*a*). Between the arrows 5 μg noradrenaline was infused slowly for 10 min into the jugular vein. After infusion had stopped and after the rise of blood pressure which it caused had subsided, the intravenous injection of eserine now caused a significant rise in blood pressure, as shown in (*b*). Two hours after infusion of noradrenaline the response to eserine again disappeared, as shown in (*c*).

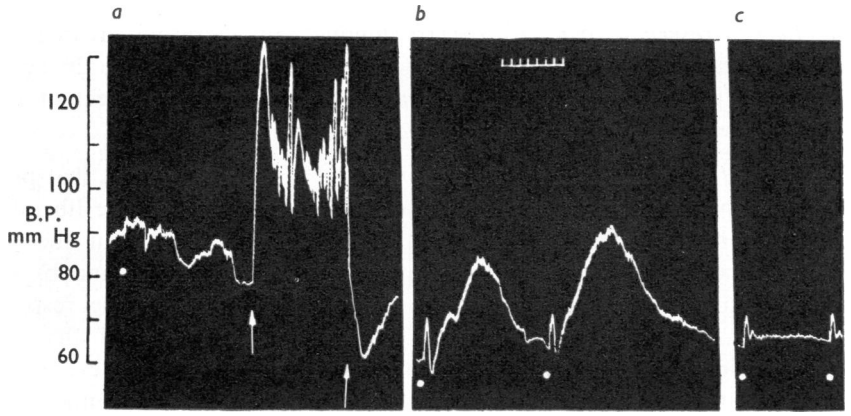

Fig. 1. The effect of eserine and slow intravenous infusion of noradrenaline on the blood pressure in a reserpine-treated rat (240 g). At white dots: 10 μg eserine intravenously. Between the arrows: slow intravenous infusion of 5 μg noradrenaline for 10 min. Records in (*b*) and (*c*) were taken 25 and 120 min respectively after infusion of noradrenaline. Time: 1 min.

In 2 reserpine-treated animals the brain and spinal cord were destroyed. Infusion of noradrenaline in these 2 experiments did not restore the response to eserine.

The effect of reserpine and dihydroxyphenylalanine on the pressor response to eserine. The slow intravenous infusion of dihydroxyphenylalanine in reserpine-treated animals also did not significantly change the response to eserine. In 7 experiments 3 mg of dihydroxyphenylalanine was slowly infused into the jugular vein of the reserpine-treated animals. Only in 2 out of 7 experiments was the hypertensive response to eserine restored after infusion of dihydroxyphenylalanine. In one experiment 6 mg of dihydroxyphenylalanine was infused, but the response to eserine did not reappear. Fig. 2 shows an experiment in which infusion of dihydroxyphenylalanine restored the response to eserine. The usual response to eserine in the reserpine-treated animal is shown in (*a*). Between (*a*) and (*b*) 2.5 mg dihydroxyphenylalanine was infused intravenously for 12 min and the record in (*b*) was taken 30 min thereafter. The slight hypertensive response to eserine gradually disappeared after 3 hr, as shown in (*c*).

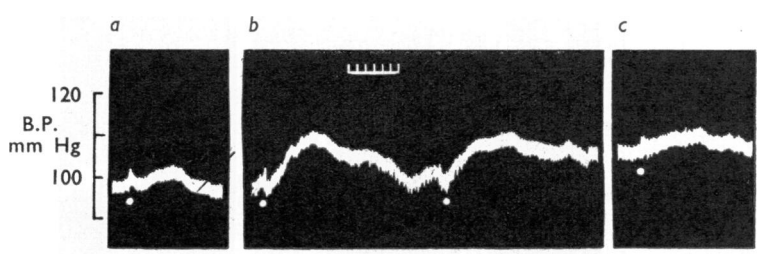

Fig. 2. The effect of eserine and slow intravenous infusion of dihydroxyphenylalanine on the blood pressure in a reserpine-treated rat (160 g). At white dots: 20 μg eserine intravenously. Between (*a*) and (*b*) 2.5 mg of dihydroxyphenylalanine was infused intravenously for 12 min. Records in (*b*) and (*c*) were taken 30 and 180 min respectively after infusion of dihydroxyphenylalanine. Time: 1 min.

The effect of reserpine and 5-hydroxytryptamine. In 7 reserpine-treated animals 5-hydroxytryptamine was infused intravenously in doses from 15 to 20 μg for 10 min. This infusion, however, did not restore the hypertensive effect of eserine. Only in 2 experiments was a slight potentiation observed.

The effect of reserpine and isopropylisoniazid. It has been found that pretreatment with isopropylisoniazid abolishes the effect of reserpine on the liberation of adrenaline from the adrenals (Kroneberg & Schümann, 1960). In the present experiments the animals were treated simultaneously by reserpine and by isopropylisoniazid in order to see whether the effect of reserpine on the response to eserine could be abolished by isopropylisoniazid. It was found that isopropylisoniazid did not abolish the inhibitory action of reserpine on the eserine hypertension. In another series of experiments isopropylisoniazid was injected after the animal was prepared for blood pressure recording. It was also found that isopropylisoniazid could not restore the hypertensive response to eserine once it was blocked by pretreatment with reserpine.

The effect of bretylium and eserine. Bretylium is a specific type of adrenergic blocking agent. It causes a specific and lasting depression of many excitatory and inhibitory responses evoked by electrical stimulation of the peripheral sympathetic nervous system, probably by impairing conduction of impulses in adrenergic neurones with consequent failure of noradrenaline and adrenaline release (Boura & Green, 1959). In our experiments bretylium abolished or significantly depressed the hypertensive response to eserine, and at the same time the hypertensive effect of adrenaline was unchanged or even potentiated. One experiment is shown in Fig. 3. The usual responses to eserine and adrenaline are shown in (*a*). Between (*a*) and (*b*) 10 mg/kg bretylium was injected intravenously and 30 min later the

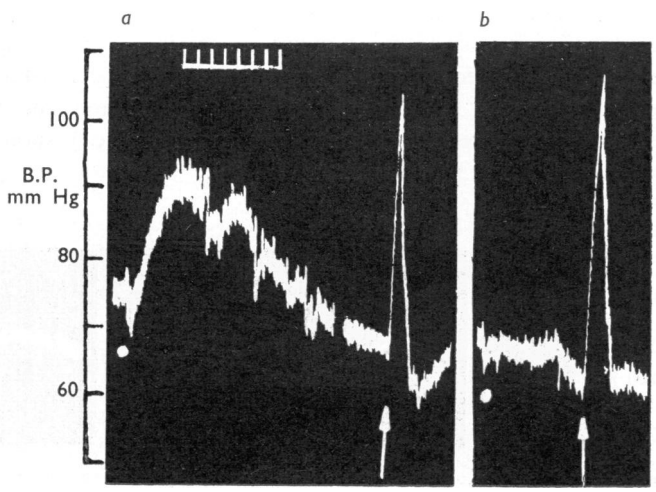

Fig. 3. The effect of eserine, adrenaline and bretylium injected intravenously on the blood pressure of the rat (190 g). At white dots: 20 μg eserine. At the arrows: 0.4 μg adrenaline. Between (*a*) and (*b*) 10 mg/kg bretylium. Record in (*b*) was taken 30 min after injection of bretylium. Time: 1 min.

response to eserine was completely blocked, while the effect of adrenaline was slightly increased, as shown in (*b*). It was also found that in doses which significantly depressed the action of eserine, bretylium did not inhibit the hypertension due to excitation of medullary centres induced by clamping the common carotid arteries.

Cocaine was found to reduce the inhibitory effect of bretylium on the hypertensive response to eserine. Boura & Green (1959) have already found that cocaine reduced the effect of bretylium on the response of the nictitating membrane to nerve stimulation. The antagonism between bretylium and cocaine on the blood pressure response to eserine is shown in Fig. 4. The usual hypertensive effect of eserine and adrenaline is shown in (*a*). Between (*a*) and (*b*) 9.2 mg/kg bretylium was injected intravenously and 30 min later the effect of eserine was abolished, while the response to adrenaline was unchanged, as shown in (*b*). Between (*b*) and (*c*) 3.7 mg/kg cocaine was injected intravenously and 10 min later the response to eserine was partly restored, as shown in (*c*).

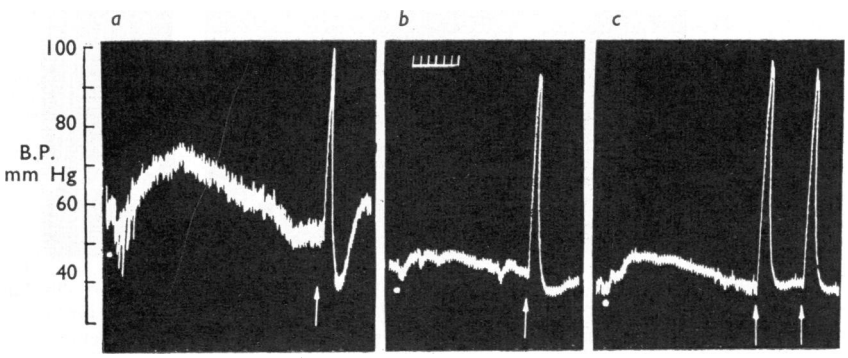

Fig. 4. The effect of eserine, adrenaline, bretylium and cocaine injected intravenously on the blood pressure of the rat (270 g). At white dots: 20 μg eserine. At the arrows: 0.4 μg adrenaline. Between (*a*) and (*b*) 9.2 mg/kg bretylium, and (*b*) was taken 30 min thereafter. Between (*b*) and (*c*) 1 mg cocaine, and (*c*) was taken 10 min thereafter. Time: 1 min.

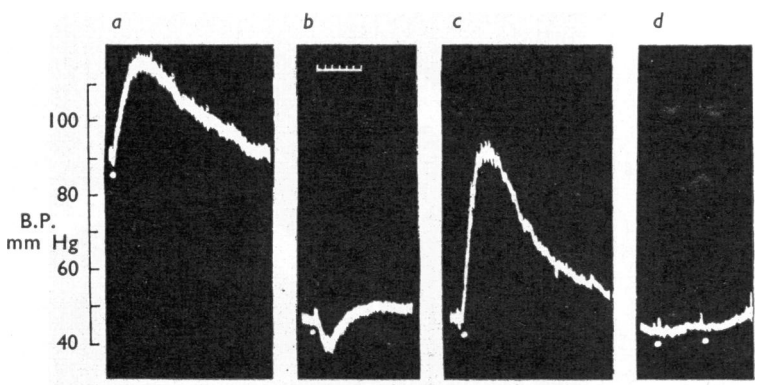

Fig. 5. The effect of hypothermia on the blood pressure response to eserine in the rat (250 g). (*a*) The response to eserine at normal body temperature. Records in (*b*) and (*d*) were taken at 21° C body temperature; (*c*) was taken at 26° C. At white dots: 20 μg eserine injected intravenously.

The effect of cooling. Following an observation of Dr. M. Miloševic (unpublished observations) that the hypertensive effect of some organophosphates was abolished by cooling, we have investigated the effect of cooling on the response to eserine. It was found that lowering of the body temperature by cooling the animal abolished completely the hypertensive effect of eserine. An experiment is shown in Fig. 5 where covering the animal with ice caused a reduction of body temperature, which was sometimes accompanied by a fall of blood pressure, as shown in Fig. 5(*b*). It can also be seen that the hypertensive effect of eserine was abolished at a body temperature of 21° C. The animal was then warmed and the body temperature rose to 26° C, at which temperature the hypertensive response to eserine reappeared, as shown in (*c*). The animal was cooled once again and the body temperature

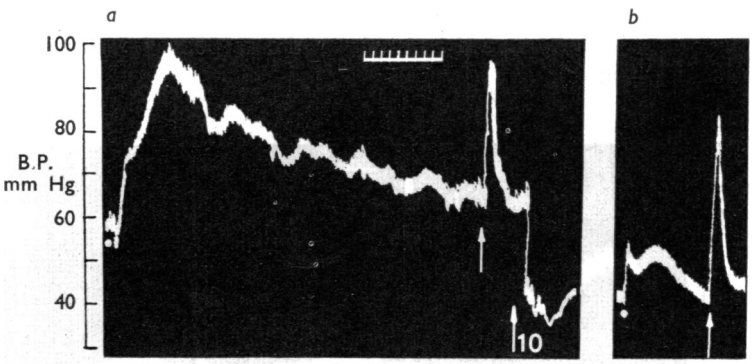

Fig. 6. The effect of eserine, noradrenaline and choline 2,6-xylyl ether bromide injected intravenously on the blood pressure of the rat (260 g). At white dots: 10 μg eserine. At the arrows: 1 μg noradrenaline. At the arrow with number 10: 7.7 mg/kg choline 2,6-xylyl ether bromide. and (*b*) was taken 34 min thereafter. Time: 1 min.

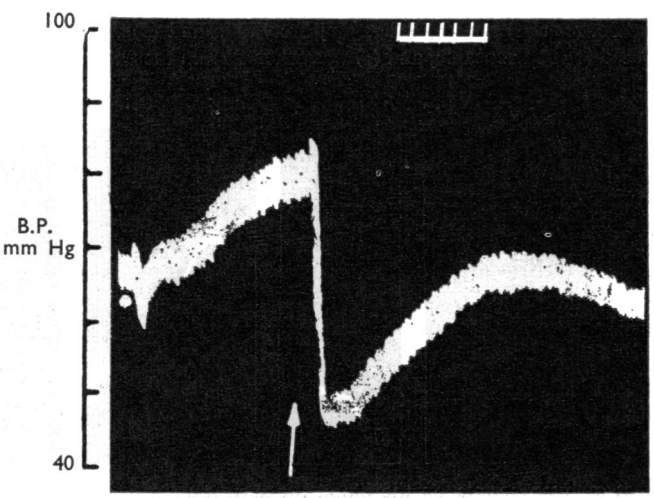

Fig. 7. The effect of eserine and choline 2,6-xylyl ether bromide injected intravenously on the blood pressure of the rat (250 g). At white dot: 20 μg eserine. At the arrow: 2 mg/kg choline 2,6-xylyl ether bromide.

dropped to 21° C with subsequent abolition of the hypertensive response to eserine, as shown in (*d*).

The effect of choline 2,6-xylyl ether bromide and eserine. In doses from 5 to 10 mg/kg choline 2,6-xylyl ether bromide significantly reduced the effect of eserine but not so readily as did bretylium. An experiment is shown in Fig. 6 where a particularly long lasting hypertensive effect of eserine is shown in (*a*). This effect can be compared with response to noradrenaline (at the first arrow). The intravenous injection of 7.7 mg/kg choline 2,6-xylyl ether bromide then caused a fall of blood pressure (at the second arrow). After 34 min the response to eserine was depressed, whereas the effect of noradrenaline was potentiated, as shown in (*b*). This effect of choline 2,6-xylyl ether bromide lasted for several hours. In one experiment choline 2,6-xylyl ether bromide was injected at the moment when the effect of eserine was still present. It was observed that the hypertensive response to eserine was blocked immediately after injection of choline 2,6-xylyl ether bromide (Fig. 7).

DISCUSSION

The present experiments show that the peripheral liberation of noradrenaline (and adrenaline) does not play a significant rôle in the hypertensive effect of eserine. In normal as in the reserpine-treated animals slow intravenous infusion of noradrenaline did not significantly alter the response to eserine. Similarly the infusion of dihydroxyphenylalanine as noradrenaline precursor in the reserpine-treated animals did not increase or restore the hypertensive effect of eserine. Thus, although eserine was found to increase the output of adrenaline from the adrenals of the cat (Stewart & Rogoff, 1921) it is unlikely that this effect occurs in the rat. The hypertensive response to eserine is different from that to tyramine. Burn & Rand (1958b) have found that the action of tyramine and substances acting like it can be restored in reserpine-treated animals by an infusion of noradrenaline into the blood stream. In the present experiments the hypertensive effect of eserine was regularly blocked by pretreatment with reserpine, but it was found that only occasionally and partially could it be restored by an intravenous infusion either of noradrenaline or dihydroxyphenylalanine.

Reserpine is known to liberate both noradrenaline and 5-hydroxytryptamine (Pletscher, Shore & Brodie, 1956; Shore & Brodie, 1957). We have now shown that the intravenous infusion of 5-hydroxytryptamine in reserpine-treated animals did not restore the hypertensive effect of eserine. It is known that pretreatment of the animal with isopropylisoniazid abolishes the effect of reserpine on the liberation of adrenaline from the adrenals (Kroneberg & Schümann, 1960). In the present experiments there was no antagonism between isopropylisoniazid and reserpine. The inhibitory effect of reserpine on the hypertensive response to eserine could not be reversed by pretreatment with isopropylisoniazid or by injection of isopropylisoniazid 30 to 180 min before eserine.

Lowering of the body temperature of the rat was found to abolish the hypertensive response to eserine. The present experiments do not allow any definite conclusion on the mechanism by which hypothermia abolishes eserine hypertension. It might be due to abolition of response of the nervous system since nervous elements

are known to be susceptible to lowering of temperature. It has also been found that the process of synaptic transmission is affected by low temperatures more readily than the process of depolarization of postsynaptic neurones (Varagic & Beleslin, 1958).

The experiments with bretylium and choline 2,6-xylyl ether bromide show that the hypertensive response to eserine is due more to adrenergic activation than to liberation of adrenaline and noradrenaline from the adrenals. Boura & Green (1959) have found that bretylium in concentrations which were able to block adrenergic neurones did not prevent the release of adrenaline and noradrenaline from the adrenal medulla by splanchnic nerve stimulation or by injection of dimethylphenylpiperazinium iodide. In the present experiments both bretylium and choline 2,6-xylyl ether bromide were found to depress or even block the hypertensive effect of eserine. Bretylium invariably had a stronger effect than choline 2,6-xylyl ether bromide. The possibility that the pressor effect of eserine might be blocked by a central action of bretylium is not supported by the experiments of Aviado & Dil (1960). They found that the intravenous injection of 2.5 to 20 mg/kg bretylium could not block the pressor response due to excitation of the medullary centres, induced either by cerebral ischaemia or by clamping the common carotid arteries. This response could be blocked, however, by combined adrenalectomy and injection of bretylium. We have now shown that bretylium, in doses which significantly depressed the pressor effect of eserine, did not inhibit the hypertension induced by clamping the common carotid arteries. It seems therefore that bretylium block of the eserine effect was most probably due to block of the adrenergic neurones. Nasmyth & Andrews (1959) have shown that cocaine antagonizes the adrenergic nerve block caused by choline 2,6-xylyl ether bromide, and Boura & Green (1959) found that cocaine antagonized the effect of bretylium. We have now shown that cocaine could partially restore the hypertensive response to eserine which was previously blocked by bretylium.

The results of the present experiments indicate that the hypertensive response to eserine in the rat is due to central activation of adrenergic nervous elements. The rôle of eserine in liberating noradrenaline and adrenaline from the adrenals and from the blood vessels is an insignificant factor in causing the hypertensive response to eserine.

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