Brit. J. Pharmacol. (1962), **19**, 451–457.

EFFECT OF GUANETHIDINE, HEMICHOLINIUM AND MEBUTAMATE ON THE HYPERTENSIVE RESPONSE TO ESERINE AND CATECHOL AMINES

BY

V. VARAGIĆ AND N. VOJVODIĆ

From the Department of Pharmacology, Medical Faculty, Belgrade, Yugoslavia

(*Received July* 23, 1962)

Guanethidine, hemicholinium and mebutamate were used to study the site and mechanism of the hypertensive response to eserine in the rat. Guanethidine was found to block very effectively the hypertensive effect of eserine and to produce at the same time a very strong potentiation of the response to catechol amines. Hemicholinium, after a certain latent period, also blocked the effect of eserine, at the same time leaving the response to adrenaline and noradrenaline intact. Mebutamate was also found to block the effect of eserine. The results of the present experiments suggest that eserine produces a central adrenergic activation in the rat.

It has been repeatedly shown that the hypertensive response to eserine in the rat is due to central stimulation (Varagić, 1955; Dirnhuber & Cullumbine, 1955; Medaković & Varagić, 1957; Lešić & Varagić, 1961; Varagić, Lešić, Vuco & Stamenović, 1961). The most probable explanation which has been suggested is a central activation of the adrenergic nervous elements. These findings have been confirmed and even used for the assessment of sympathetic function (Cass & Spriggs, 1961).

Guanethidine, like bretylium, impairs the postganglionic adrenergic transmission by preventing the liberation of the adrenergic transmitter from the nerve endings (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960; Boura & Green, 1959). Guanethidine in contrast to bretylium depletes tissue catechol amines (Cass, Kuntzman & Brodie, 1960; Sheppard & Zimmermann, 1959). Hemicholinium has been shown to inhibit the synthesis of acetylcholine (MacIntosh, Birks & Sastry, 1956; Gardiner, 1961). Gardiner (1961) concluded that hemicholinium does not inhibit choline acetylase directly but that it competes with choline for access to the enzyme through structural barriers in the cell. These substances were used in these experiments in order to get more precise information on the type of the central activation produced by eserine in the rat, and on the mechanism of that activation. Mebutamate was used because of its property of producing hypotension by an action on the brain stem vasomotor centres (Berger, Douglas, Kletzkin, Ludwig & Margolin, 1961). It was therefore expected that this substance might be useful in further elucidating the hypertensive response to eserine. The responses to eserine were also compared with the effects of adrenaline and noradrenaline in order to differentiate

the mechanisms by which guanethidine, hemicholinium and mebutamate could affect the eserine hypertension.

METHODS

Rats of both sexes (165 to 325 g) were used in these experiments. The animals were anaesthetized with 0.7 ml./100 g body weight of 25% w/v urethane solution injected subcutaneously or sometimes intraperitoneally. 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 catheter, 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 to 0.2 ml. and washed in with the same volume of isotonic sodium chloride solution.

Noradrenaline was infused intravenously by means of a Palmer slow injecting machine. The doses of infused noradrenaline ranged from 6 μg to 10 μg. The rate of infusion ranged from 625 ng/min to 1.25 μg/min.

The following drugs were used: adrenaline hydrochloride, noradrenaline bitartrate, eserine salicylate, guanethidine sulphate, hemicholinium dibromide, mebutamate (2 methyl-2-*sec*-butyl-1,3-propanediol dicarbamate) in propylene glycol and choline chloride.

RESULTS

Guanethidine and the response to eserine. It was found previously that both xylocholine and bretylium could depress or even block the hypertensive response to eserine in the rat (Lešić & Varagić, 1961). In the present experiments guanethidine was found to block this effect of eserine as well and at the same time to potentiate the effects of adrenaline and noradrenaline. One experiment is shown in Fig. 1. The control responses to eserine (0.08 mg/kg), adrenaline (6 μg/kg) and noradrenaline (6 μg/kg) are shown in A. Between A and B guanethidine was injected intravenously in a dose of 8 mg/kg, and 15 min later the response to eserine was

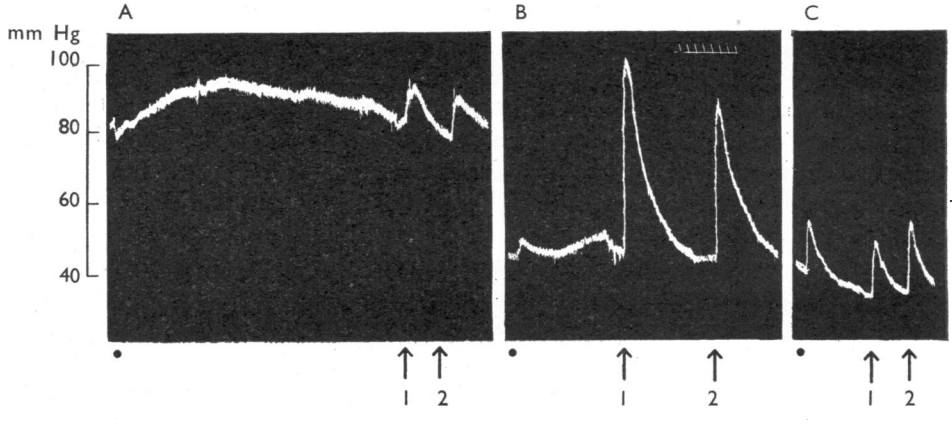

Fig. 1. The effect of guanethidine on the blood pressure responses to eserine and catechol amines in the rat under urethane, 250 g. At dots, 20 μg eserine intravenously. At the arrow 1, 1.5 μg adrenaline i.v. At the arrow 2, 1 μg noradrenaline i.v. Between A and B, 2 mg guanethidine i.v. Between B and C, slow infusion of 10 μg noradrenaline i.v. Time, 1 min intervals.

blocked whereas the effects of adrenaline and noradrenaline were markedly potentiated, as shown in B. In order to determine whether potentiation of the responses to catechol amines was connected with the ability of guanethidine to deplete the stores of catechol amines, an experiment was done in which a slow infusion of noradrenaline was given. In this experiment 10 μg noradrenaline was slowly infused between B and C, the rate of infusion being 1.2 μg/min. After the blood pressure rise caused by the infused noradrenaline had returned to normal, the responses to adrenaline and noradrenaline were now almost the same as before injection of guanethidine, as shown in C. However, the slow infusion of noradrenaline did not restore the prolonged pressor response to eserine which is also shown in C.

Hemicholinium and the response to eserine. Hemicholinium blocked the hypertensive effect of eserine, but only after a latent period. Fig. 2 shows an experiment in which 27 min elapsed after the injection of hemicholinium before the effect of

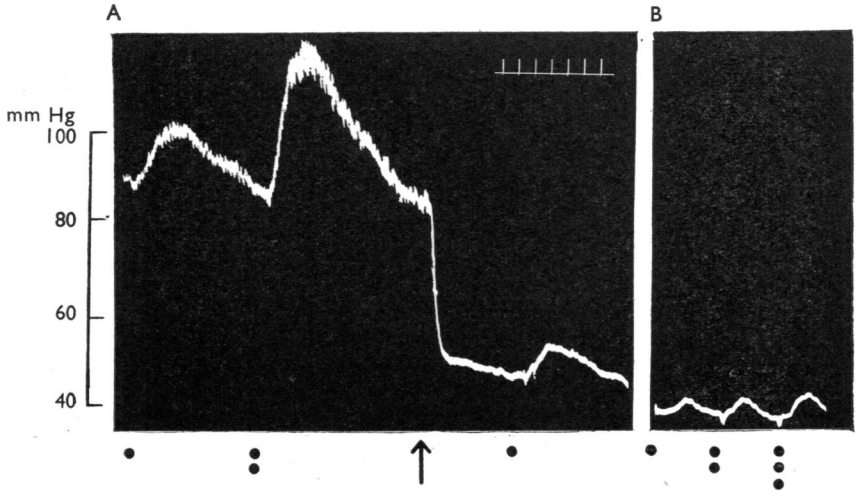

Fig. 2. The effect of hemicholinium on the blood pressure response to eserine in the rat under urethane, 260 g. At one dot, 40 μg eserine intravenously. At two dots, 100 μg eserine i.v. At three dots, 200 μg eserine i.v. At the arrow, 2 mg hemicholinium i.v. Between A and B, 15 min. Time, 1 min intervals.

eserine was blocked. The control responses to 0.153 mg/kg eserine (at one dot) and to 0.384 mg/kg eserine (at two dots) are shown in A. At the arrow hemicholinium was injected in a dose of 7.46 mg/kg. The animal was kept on artificial respiration because hemicholinium depresses respiratory movements (Long & Schueler, 1954; Longo, 1959; Holmes & Wilson, 1960). Seven minutes after injection of hemicholinium the effect of eserine was depressed, as shown in A. B shows the record 27 min after injection of hemicholinium and when even a dose of 0.768 mg/kg eserine did not cause a significant effect on the blood pressure.

It has been found that choline chloride reversed some effects of hemicholinium (Long & Schueler, 1954). In the present experiments choline chloride was injected in doses up to 16 mg/kg, but did not restore the hypertensive effect of eserine once it was blocked by hemicholinium.

Fig. 3 shows the effects of hemicholinium on the pressor responses to eserine, adrenaline and noradrenaline. Unlike guanethidine, hemicholinium did not

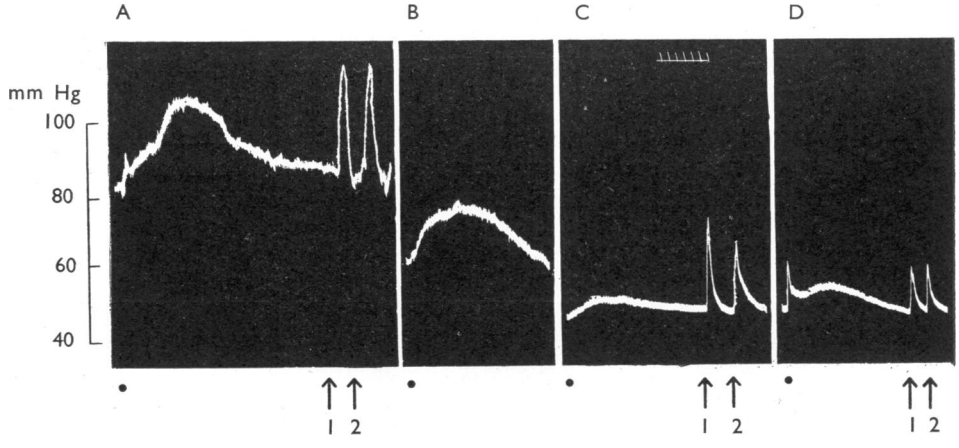

Fig. 3. The effect of hemicholinium on the blood pressure responses to eserine and catechol amines in the rat under urethane, 250 g. At dots, 40 μg eserine intravenously. At the arrow 1, 0.6 μg adrenaline intravenously. At the arrow 2, 0.6 μg noradrenaline intravenously. Between A and B, 1.5 mg hemicholinium intravenously. Between B and C, the same dose of hemicholinium was repeated. B was recorded 44 min after the first injection of hemicholinium, C was recorded 20 min after the second dose of hemicholinium. Between C and D, slow infusion of 10 μg noradrenaline. Time, 1 min intervals.

potentiate the responses to adrenaline and noradrenaline. The control responses to eserine (0.16 mg/kg), adrenaline (2.4 μg/kg) and noradrenaline (2.4 μg/kg) are shown in A. Between A and B, 6 mg/kg hemicholinium was injected and 44 min later (in B) the effect of eserine was depressed. Between B and C another dose of 6 mg/kg hemicholinium was injected and 20 min later (in C) the response to eserine was almost blocked, whereas the effects of adrenaline and noradrenaline were depressed. Between C and D 10 μg noradrenaline was slowly infused. The infusion did not restore the effect of eserine and it produced further inhibition of the responses to catechol amines.

Mebutamate and the response to eserine. Mebutamate was found to block the hypertensive effect of eserine, and an experiment is illustrated in Fig. 4. The control responses to eserine (0.16 mg/kg), adrenaline (2.4 μg/kg) and noradrenaline (2.4 μg/kg) are shown in A. Between A and B 20 mg/kg mebutamate was injected, and 13 min later the responses to eserine and catechol amines were not significantly changed. Between B and C, two doses of mebutamate, 20 mg/kg each, were

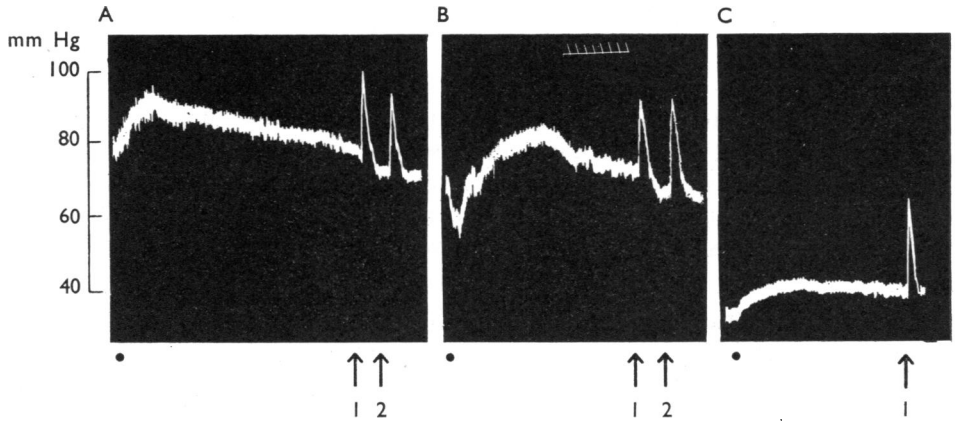

Fig. 4. The effect of mebutamate on the blood pressure responses to eserine and catechol amines in the rat under urethane, 250 g. At dots, 40 μg eserine intravenously. At the arrow 1, 0.6 μg adrenaline i.v. At the arrow 2, 0.6 μg noradrenaline i.v. Between A and B, 5 mg mebutamate i.v. Between B and C, 10 mg mebutamate (in two doses) i.v. Time, 1 min intervals.

injected, and 5 min after the last injection the effect of eserine was almost blocked, whereas the response to adrenaline was unaltered.

DISCUSSION

Guanethidine very effectively blocked the hypertensive response to eserine in the rat, causing at the same time a very strong potentiation of the response to catechol amines. It has been found previously that both bretylium and xylocholine significantly depress or even block the hypertensive effect of eserine, whereas the effect of adrenaline is unchanged or potentiated (Lešić & Varagić, 1961). It is now generally accepted that both bretylium and guanethidine impair postganglionic adrenergic transmission and do not antagonize the actions of adrenaline and noradrenaline at the effector site (Boura & Green, 1959 ; Maxwell, Mull & Plummer, 1959 ; Maxwell, Plummer, Povalski & Schneider, 1960). In the present experiments with guanethidine the hypertensive response to eserine in the rat may be due to central activation of the adrenergic nervous elements.

The ability of guanethidine to cause a marked potentiation of the responses to catechol amines, while blocking the effect of eserine, is probably due to another property of this substance. Guanethidine, in contrast to bretylium, depletes tissue catechol amine levels (Cass, Kuntzman & Brodie, 1960 ; Sheppard & Zimmermann, 1959). Depletion of catechol amine content by reserpine has already been found to potentiate the action of adrenaline and noradrenaline (Burn & Rand, 1958). It is therefore possible that guanethidine potentiates the responses to catechol amines by decreasing the stores of noradrenaline in the blood vessels. This is supported by our experiments with the intravenous infusion of noradrenaline. When a slow intravenous infusion of noradrenaline is given during a period of 8 to 16 min, which

probably results in a building up of a store in the vessel wall, then when the effect of the infused noradrenaline on the blood pressure has passed off, the pressor effects of injected adrenaline and noradrenaline are similar to those before injection of guanethidine. Potentiation of the responses to catechol amines might depend on other actions of guanethidine. Cocaine and reserpine are known to block the noradrenaline uptake by the tissue and thus increase the amount of noradrenaline available for combination with its tissue receptors (Muscholl, 1961). Further experiments are necessary in order to prove this possibility in the case of guanethidine.

Hemicholinium was also found to block the hypertensive response to eserine, but, unlike guanethidine, it did not potentiate the effects of adrenaline and noradrenaline. This finding might to some extent indicate the cholinergic nature of activation by eserine in the central nervous system of the rat. However, it is difficult to accept that the toxic effects of hemicholinium are due to its action on the central nervous system, because it is unlikely that a bisquaternary ammonium compound of this type would penetrate sufficiently through the blood-brain barrier (Schueler, 1955; Kase & Borison, 1958; Gardiner, 1961). In the present experiments rather high doses of hemicholinium were necessary in order to block the pressor effect of eserine. Once it was blocked by hemicholinium, it was impossible to restore the effect of eserine by choline chloride. It would therefore mean that hemicholinium might have some other actions by which the hypertensive response to eserine could be blocked. These actions are different from those of guanethidine because hemicholinium does not affect the responses to catechol amines.

Since mebutamate, which lowers blood pressure by a central mechanism (Berger *et al.*, 1961), depressed or blocked the hypertensive effect of eserine in the rat, it was inferred that this provided further evidence that eserine produced a rise of blood pressure by a central action.

The authors are very indebted to Dr M. J. Rand, from the School of Pharmacy, University of London, and to Dr J. P. Long, from the Department of Pharmacology, State University of Iowa, for supplying hemicholinium; to Ciba for supplying guanethidine ("Ismeline"); and also to Professor Dr P. Štern, from the Department of Pharmacology, Medical Faculty, Sarajevo, for supplying mebutamate.

REFERENCES

BERGER, F. M., DOUGLAS, J. F., KLETZKIN, M., LUDWIG, B. J. & MARGOLIN, S. (1961). The pharmacological properties of 2-methyl-2-sec-butyl-1,3-propanediol dicarbamate (Mebutamate, W-583), a new centrally acting blood pressure lowering agent. *J. Pharmacol. exp. Ther.*, **134**, 356–365.

BOURA, A. L. A. & GREEN, A. F. (1959). The actions of bretylium: adrenergic neurone blocking and other effects. *Brit. J. Pharmacol.*, **14**, 536–548.

BURN, J. H. & RAND, M. J. (1958). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol. (Lond.)*, **144**, 314–336.

CASS, R., KUNTZMAN, R. & BRODIE, B. B. (1960). Norepinephrine depletion as a possible mechanism of action of guanethidine (SU 5864), a new hypotensive agent. *Proc. Soc. exp. Biol., N.Y.*, **103**, 871–872.

CASS, ROSEMARY & SPRIGGS, T. L. B. (1961). Tissue amine levels and sympathetic blockade after guanethidine and bretylium. *Brit. J. Pharmacol.*, **17**, 442–450.

CONDON, N. E. (1951). A modification of the conventional mercury manometer for blood pressure recordings. *Brit. J. Pharmacol.*, **6**, 19–20.

DIRNHUBER, P. & CULLUMBINE, H. (1955). The effect of anticholinesterase agents on the rat's blood pressure. *Brit. J. Pharmacol.*, **10**, 12–15.

GARDINER, J. E. (1961). The inhibition of acetylcholine synthesis in brain by a hemicholinium. *Biochem. J.*, **81**, 297–303.

HOLMES, R. & WILSON, H. (1960). Action of hemicholinium compound (HC_3) on the central inspiratory discharge and diaphragm activity in the cat. *J. Physiol. (Lond.)*, **153**, 62–63*P*.

KASE, Y. & BORISON, H. L. (1958). Central respiratory depressant action of hemicholinium in the cat. *J. Pharmacol. exp. Ther.*, **122**, 215–233.

LEŠIĆ, R. & VARAGIĆ, V. (1961). Factors influencing the hypertensive effect of eserine in the rat. *Brit. J. Pharmacol.*, **16**, 99–107.

LONG, J. P. & SCHUELER, F. W. (1954). A new series of cholinesterase inhibitors. *J. Amer. pharm. Ass.*, **43**, 79–87.

LONGO, V. G. (1959). Action of hemicholinium No. 3 on phrenic nerve action potentials. *Arch. int. Pharmacodyn.*, **119**, 1–9.

MACINTOSH, F. C., BIRKS, R. J. & SASTRY, P. B. (1956). Pharmacological inhibition of acetylcholine synthesis. *Nature (Lond.)*, **178**, 1181.

MAXWELL, R. A., MULL, R. P. & PLUMMER, A. J. (1959). (2-(Octahydro-1-azocinil)-ethyl)-guanidine sulfate (CIBA 5864-SU), a new synthetic antihypertensive agent. *Experientia (Basel)*, **15**, 267.

MAXWELL, R. A., PLUMMER, A. J., POVALSKI, H. & SCHNEIDER, F. (1960). Concerning a possible action of guanethidine (SU-5864) in smooth muscle. *J. Pharmacol. exp. Ther.*, **129**, 24–30.

MAXWELL, R. A., PLUMMER, A. J., SCHNEIDER, F., POVALSKI, H. & DANIEL, A. I. (1960). Pharmakologie von Guanethidin einer blutdrucksenkend wirkenden Substanz mit specifischer peripherer Sympaticushemmung. *Schweiz. med. Wschr.*, **90**, 109–113.

MEDAKOVIĆ, M. & VARAGIĆ, V. (1957). The effect of eserine and neostigmine on the blood pressure of conscious rats. *Brit. J. Pharmacol.*, **12**, 24–27.

MUSCHOLL, E. (1961). Effect of cocaine and related drugs on the uptake of noradrenaline by heart and spleen. *Brit. J. Pharmacol.*, **16**, 352–359.

SCHUELER, F. W. (1955). A new group of respiratory paralyzants: I. The "Hemicholiniums." *J. Pharmacol. exp. Ther.*, **115**, 127–143.

SHEPPARD, H. & ZIMMERMANN, J. (1959). Effect of guanethidine (SU-5864) on tissue catecholamines. *Pharmacologist*, **1**, 69.

VARAGIĆ, V. (1955). The action of eserine on the blood pressure of the rat. *Brit. J. Pharmacol.*, **10**, 349–353.

VARAGIĆ, V., LEŠIĆ, R., VUCO, J. & STAMENOVIĆ, B. (1961). The effect of eserine on the activity of adrenergic nerves in the rat. *Biochem. Pharmacol.*, **8**, 10.