Br. J. Pharmac. (1969), 35, 265–270.

Evidence for muscarinic receptors in the adrenal medulla of the dog

S. O. KAYAALP* AND R. K. TÜRKER

Departments of Pharmacology, Hacettepe and Ankara Universities, Ankara, Turkey.

1. The release of catecholamines from the adrenal medulla by the cholinergic drugs was monitored by the perfusion pressure rise in autoperfused and sympathetically denervated hindlimbs of the dog.

2. Acetylcholine, dimethylphenylpiperazinium (DMPP) and methacholine, given in relatively small doses into the aorta proximal to the blood supply to the adrenal glands, caused a marked rise in perfusion pressure which was due to the release of catecholamines from the adrenal medulla.

3. Atropine (1 mg/kg intravenously) blocked the pressor response to methacholine only. The ganglion-blocking agents (mecamylamine 4–5 mg/kg or hexamethonium 10 mg/kg) given subsequently blocked the pressor responses to the other two cholinergic drugs. The ganglion-blocking agents, when given before atropine, blocked the pressor action only of DMPP. These agents partly depressed, rather than potentiated, the pressor response to methacholine.

4. The results suggest that muscarinic as well as nicotinic receptors are present in the adrenal medulla of the dog.

5. Neither atropine nor ganglion-blocking drugs alone reduced the pressor response to acetylcholine. It is postulated that the blockade of one set of receptors makes more acetylcholine available for the other set of receptors and so inactivation of the former receptors are compensated by the increased release through the activation of the latter's.

Although a number of studies are available concerning the presence of both nicotinic and muscarinic cholinergic receptors and their interaction with drugs in sympathetic ganglia, the response of muscarinic cholinergic receptors in the adrenal medulla to drugs has been studied less. It was shown by Feldberg, Minz & Tsudzimura (1934) that in the cat the action of acetylcholine, as well as of splanchnic stimulation, on the adrenal medulla is mediated principally through the nicotinic receptors, but that a minor muscarinic component which is abolished by small doses

* On leave of absence from the Hacettepe University. Present address: Department of Pharmacology, State University of New York at Buffalo, 122 Capen Hall, Buffalo, New York 14214.

of atropine was also involved in this action. Douglas & Poisner (1965) confirmed the presence of two sets of acetylcholine receptors in the cat adrenal medulla and showed that muscarinic agents release adrenaline preferentially from the gland.

In the present work an attempt was made to study the *in vivo* interaction between the cholinergic and anticholinergic agents in the adrenal medulla of the dog. Evidence will be presented that muscarinic receptors, as well as nicotinic receptors, are involved in the catecholamine release by cholinergic drugs.

Methods

Twenty mongrel dogs of both sexes, weighing from 10 to 18 kg, were used. They were anaesthetized by intravenous injection of sodium pentobarbitone 30 mg/kg. The trachea was cannulated to secure a free airway and the left jugular vein was cannulated for intravenous injection of the drugs.

The method used for studying the effect of the drugs on the release of catecholamines from the adrenal medulla has been described in detail elsewhere (Kayaalp, 1968), so only a brief account will be presented here. The hindquarters were autoperfused with a Sigmamotor pump interposed by means of appropriate tubing between the proximal and distal portions of the aorta, tied at mid-lumbar level, as described by Beck (1961). In some experiments perfusion was made from the left carotid artery, rather than the proximal aorta, to the distal aorta. The vasculature of the hindquarters was acutely denervated by removing two adjacent sympathetic ganglia in the mid-lumbar level on each side. This caused a complete or near complete sympathetic denervation in the perfused area as evidenced during the experiment by a lack of either the reflex dilation in response to a rise in systemic blood pressure elicited by the intravenous administration of noradrenaline or the pressor response to the bilateral carotid occlusion. The denervated vasculature served as a non-specific catecholamine detector of adequate sensitivity. The rate of the perfusion pump was kept constant throughout the experiment. Perfusion pressure in the hindquarters was monitored on smoked paper from a single-arm mercury manometer attached to a side arm of the inflow cannula, whereas systemic blood pressure was monitored from a double arm mercury manometer attached to a side arm of the cannula inserted into the proximal portion of the aorta, or left carotid artery.

A polyethylene cannula of approximately 1 mm outside diameter was placed retrogradely, through one of its lumbar branches, into the proximal aorta in such a way that the tip lay proximal to the blood supply to the adrenal glands. The occurrence of a pressor response to small doses of dimethylphenylpiperazinium given through the catheter was taken as evidence that the tip lay at the intended level.

Drugs were injected through the cannula in a volume of about 0.8 ml. and washed in with 0.2 ml. physiological saline while the perfusion pump was shut off. The pump was kept off for an additional period of about 5 sec after the administration of the drug. The total period during which the pump was off was less than 15 sec. The pump was turned off for two reasons: first, to avoid as much as possible the passage of the drug into the perfused area and, second, to enable more drug to reach the adrenal glands. In the experiments in which perfusion was established from the carotid artery to the distal aorta the interruption of the perfusion was not necessary during the intra-aortic administration of the drugs.

The significance of the difference between the means of the responses was evaluated by Student's *t* test. A *P* value of less than 0.05 was considered significant.

The following drugs were used: acetylcholine chloride, dimethylphenylpiperazinium iodide (DMPP), methacholine chloride, noradrenaline bitartrate, mecamylamine hydrochloride, synthetic bradykinin (Sandoz) and hexamethonium bromide. Doses of the drugs were expressed as the salt, except for acetylcholine and noradrenaline.

Results

Acetylcholine (10 μg/kg), DMPP (3–5 μg/kg) and methacholine (10 μg/kg), each given intra-aortally, produced a sharp rise in perfusion pressure which is due to the release of catecholamines from the adrenal medulla as previously reported

TABLE 1. *Effect of atropine (1 mg/kg i.v.) and the ganglion blocking agents (hexamethonium 10 mg/kg. or mecamylamine 4–5 mg/kg i.v.) given subsequently on the pressor responses to acetylcholine (10 μg/kg), DMPP (3–5 μg/kg) and methacholine (10 μg/kg), each given intra-aortally, and to intra-arterial noradrenaline (0·6 μg)*

	Responses to			
	Acetylcholine	DMPP	Methacholine	Noradrenaline
Control	48±6* (12)	74±8 (11)	36±3 (8)	51±5 (12)
After atropine	50±7 (12)	86±9 (11)	2±3 (8)	53±5 (12)
After ganglion blocking agent	6±2 (9)	8±3 (9)	—	54±6 (9)

* Mean ± standard error of the mean. Figures in parenthesis refer to the number of experiments.

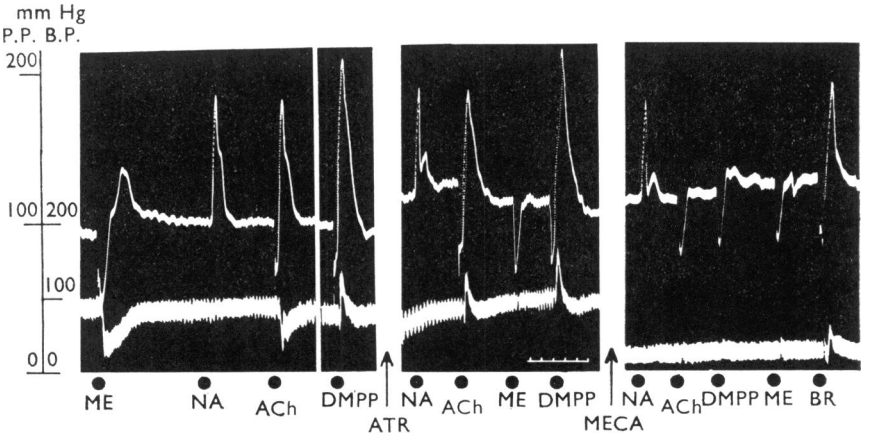

FIG. 1. Effect of atropine (1 mg/kg i.v.) (ATR) and mecamylamine (4 mg/kg i.v.) (MECA), given subsequently, on the pressor responses to methacholine (10 μg/kg) (ME), acetylcholine (10 μg/kg) (ACh), dimethylphenylpiperazinium (5 μg/kg) (DMPP), bradykinin (0.5 μg/kg) (BR), each given into the aorta, and to noradrenaline (0.6 μg) (NA) given intra-arterially into the perfused area. Dog anaesthetized with pentobarbital and hindquarters autoperfused under constant output. Note that perfusion pressure (upper trace) was recorded through a single arm manometer and systemic blood pressure (lower trace) through a double arm manometer. Time marks, 1 min.

(Kayaalp, 1968). The two choline esters caused a simultaneous fall in systemic blood pressure. Since the circulation to the perfused area was arrested during the intra-aortic administration of the drugs, a fall in perfusion pressure by artefact was observed in the experiments with perfusion of blood from proximal to distal aorta. This fall usually lasted less than 1 min, but it was usually longer when methacholine was injected, suggesting a minor vasodilation due to the recirculation of this drug. The responsiveness of the perfused vascular bed was monitored throughout the experiment by injection of 0.6 μg noradrenaline intra-arterially (into the tubing at a point distal to the Sigamotor pump).

In twelve dogs atropine (1 mg/kg intravenously) was injected after obtaining the control responses to the test drugs mentioned above (Fig. 1). The results are summarized in Table 1. Atropine abolished almost completely the pressor response to methacholine. It caused a slight increase in the pressor responses elicited by acetylcholine and DMPP ; however, the differences were not statistically significant. Atropine did not affect the responsiveness of the perfused vascular bed to intra-arterial noradrenaline.

The subsequent injection of the ganglion-blocking drugs, mecamylamine (4–5 mg/kg intravenously) or hexamethonium (10 mg/kg intravenously), blocked almost completely the pressor responses elicited by acetylcholine and DMPP given intra-aortally, at a time when there was no marked change in the pressor response to intra-arterial noradrenaline. Following the administration of the ganglion blocking agents in addition to atropine, the responsiveness of the adrenal medulla to non-cholinergic stimulation was found adequate as evidenced by obtaining a marked pressor response upon the intra-aortic injection of bradykinin (0.3–1 μg/kg) in some experiments.

In eight additional experiments, the order of administration of the blocking drugs was changed so that the ganglion-blocking agent was injected before atropine. The results are summarized in Table 2. After the administration of the ganglion-blocking agent, the pressor response to DMPP was inhibited greatly. The pressor response elicited by methacholine was reduced to approximately 65% of the control. Interestingly, the pressor response to acetylcholine was increased by about 30%. These changes did not, however, seem to be significant. An increase of similar magnitude to that observed in the responses to acetylcholine occurred in the responsiveness of the perfused vascular bed to intra-arterial noradrenaline. The subsequent administration of atropine (1 mg/kg intravenously) blocked completely the pressor responses to acetylcholine and methacholine.

TABLE 2. *Effect of the ganglion-blocking agents (hexamethonium 10 mg/kg or mecamylamine 4–5 mg/kg i.v.) and atropine (1 mg/kg i.v.) given subsequently on the pressor responses to acetylcholine (10 μg/kg), DMPP (3–5 μg/kg) and methacholine (10 μg/kg), each given intra-aortally, and to intra-arterial noradrenaline (0·6 μg).*

	Responses to			
	Acetylcholine	DMPP	Methacholine	Noradrenaline
Control	36±7* (8)	48±10 (7)	34±6 (5)	41±5 (8)
After ganglion blocking agent	47±5 (8)	4±2 (7)	22±7 (5)	50±7 (8)
After atropine	4±2 (6)	1±3 (5)	2±1 (4)	48±8 (6)

* Mean ± standard error of the mean. Figures in parenthesis refer to the number of experiments.

Discussion

The results obtained in the present work suggest the presence of two types of cholinergic receptors in the adrenal medulla of the dog. Thus they confirm the similar results obtained in the cat (Feldberg *et al.*, 1934 ; Douglas & Poisner, 1965). One of the receptor types was activated selectively by methacholine and blocked by atropine. Therefore these receptors have the characteristics of the muscarinic receptors. The other type of cholinergic receptors, which were selectively activated by DMPP and blocked by hexamethonium and mecamylamine, are the nicotinic receptors of the adrenal medulla. While working in dogs treated by atropine, as usually done in the previous works on this subject, they are the only cholinergic receptors of the adrenal medulla available for the cholinergic agents.

Neither atropine nor ganglion-blocking drugs alone could block the catecholamine release by acetylcholine. They had to be given in combination in order to block the response to acetylcholine. This suggests that acetylcholine activates both types of cholinergic receptors of the adrenal medulla. There seem, however, to be some peculiarities in the action of acetylcholine on the adrenal medulla. Atropine or the ganglion-blocking agents alone did not cause any inhibition of the pressor response to acetylcholine. It may be postulated that the blockade of one set of cholinergic receptors in the adrenal medulla makes available more acetylcholine for the other set of receptors. Thus the increase in the release through the activation of one set of receptors compensates the blockade of the other set of receptors. A parallel observation was made in the *in situ* canine and cat ileum preparation by several investigators (Ahlquist & Levy, 1959 ; Levy, 1959 ; Türker, Kiran & Kaymakcalan, 1965) who usually could not obtain any marked inhibition of the relaxing effect of adrenaline on the ileal motility after the blockade of α or β adrenoceptive receptors alone. This effect of adrenaline is mediated through the simultaneous activation of both types of adrenoceptive receptors. This phenomenon resembles the catecholamine release from the adrenal medulla by acetylcholine in that the activation of two different populations of receptors leads to the same general effect.

There was a reduction of the pressor response to methacholine after the administration of ganglion-blocking agents. It is possible that the large fall in blood pressure elicited by methacholine facilitates, through the baroreceptor reflex mechanism, the direct catecholamine releasing action of this drug. Indeed, the fall in the systemic blood pressure was reported to increase the release of catecholamines from the adrenal medulla (Booker, Fisher, Coffey & Linares, 1962 ; De Schaepdryver, 1959). So the partial inhibition of the release by methacholine after the ganglion-blocking agent might be due to the elimination of this facilitation rather than to the blockade of the receptors involved in the methacholine action. It has recently been shown that methacholine caused a marked release of adrenal catecholamines in spinal dogs when given intra-aortally (Kayaalp & McIsaac, 1968). This observation and resistance to ganglionic blockers of the response to methacholine, as observed in the present work, are against the possibility that the action of methacholine on the adrenal medulla may be due completely to the reflex activated by the fall in blood pressure.

The ganglion-blocking agents have been demonstrated to potentiate, rather than to depress, the rise in blood pressure produced by the systemic injection of the muscarinic compounds like pilocarpine, McN-A-343 and AHR-602 (Root, 1951 ; Trendelenburg, 1955 ; Roszkowski, 1961 ; Levy & Ahlquist, 1962 ; Franko, Ward & Alphin,

1963). This pressor response has been found to be due chiefly to the stimulation of sympathetic ganglia. The reduction of the catecholamine release by methacholine after the ganglionic-blocking agents, as found in the present work, therefore suggests that the interaction between the ganglion-blocking drugs and muscarinic receptors may be different in the adrenal medulla from that in the sympathetic ganglia.

This investigation was supported in part by NATO Research Grant No. 212. We would like to thank the firms who supplied us with dimethylphenylpiperazinium (Parke, Davis and Co., Ann Arbour), hexamethonium (Squibb, New York), norepinephrine (Hoechst, Frankfurt) and synthetic bradykinin (Sandoz, Basle). Thanks are also due to Dr. R. J. McIsaac for the examination of the manuscript. The valuable technical assistance by Messrs. R. Sarihüseyinoglu and B. Koc was greatly appreciated. This paper was presented in part at the Meetings of FASEB at Atlantic City, New Jersey, April 15–20, 1968.

REFERENCES

AHLQUIST, R. P. & LEVY, B. (1959). Adrenergic receptive mechanism of canine ileum. *J. Pharmac. exp. Ther.*, **127**, 146–149.

BECK, L. (1961). Active reflex dilatation in the innervated perfused hind leg of the dog. *Am. J. Physiol.*, **201**, 123–128.

BOOKER, W. M., FISHER, E., COFFEY, W. & LINARES, R. (1962). Studies on the secretion of catecholamines during carotid sinus reflex involvement. *Archs int. Pharmac. exp. Thér.*, **139**, 336–345.

DE SCHAEPDRYVER, A. F. (1959). Physio-pharmacological effects on suprarenal secretion of adrenaline and noradrenaline in dogs. *Archs int. Pharmac. exp. Thér.*, **121**, 222–253.

DOUGLAS, W. W. & POISNER, A. M. (1965). Preferential release of adrenaline from the adrenal medulla by muscarine and pilocarpine. *Nature, Lond.*, **208**, 1102–1103.

FELDBERG, W., MINZ, B. & TSUDZIMURA, H. (1934). The mechanism of the nervous discharge of adrenaline. *J. Physiol., Lond.*, **81**, 286–304.

FRANKO, B. V., WARD, J. W. & ALPHIN, R. S. (1963). Pharmacologic studies of N-benzyl-3-pyrrolidyl acetate methobromide (AHR-602), a ganglionic stimulating agent. *J. Pharmac. exp. Ther.*, **139**, 25–30.

KAYAALP, S. O. (1968). A method for studying the effect of drugs on the catecholamine release from the adrenal medulla in the dog. *Arzneimittel-Forsch.*, **18**, 84–87.

KAYAALP, S. O. & MCISAAC, R. J. (1968). *In vivo* release of catecholamines from the adrenal medulla by selective activation of cholinergic receptors. *Archs int. Pharmac. exp. Thér.*, in the Press.

LEVY, B. (1959). Adrenergic blockade produced by the dichloro analogs of epinephrine, arterenol and isoproterenol. *J. Pharmac. exp. Ther.*, **127**, 150–156.

LEVY, B. & AHLQUIST, R. P. (1962). A study of sympathetic ganglionic stimulants. *J. Pharmac. exp. Ther.*, **137**, 219–228.

ROOT, M. A. (1951). Certain aspects of the vasopressor action of pilocarpine. *J. Pharmac. exp. Ther.*, **101**, 125–131.

ROSZKOWSKI, A. P. (1961). An unusual type of sympathetic ganglionic stimulant. *J. Pharmac. exp. Ther.*, **132**, 156–170.

TRENDELENBURG, U. (1955). The potentiation of ganglionic transmission by histamine and pilocarpine. *J. Physiol., Lond.*, **129**, 337–351.

TÜRKER, K., KIRAN, B. K. & KAYMAKCALAN, S. (1965). Distribution of adrenergic receptors in the cat ileum. *Archs int. Pharmac. exp. Thér.*, **156**, 130-142.

(*Received July* 2, 1968)