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Muscarinic component of splanchnic-adrenal transmission in the dog

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1. The effect of atropine on catecholamine release evoked by stimulation of the splanchnic nerve was studied in the adrenal medulla of the dog. The magnitude of the catecholamine release was estimated biologically on the basis of the pressor response occurring in the perfused and acutely sympathectomized hindquarters of the same dog by comparing it with responses elicited by intravenous injection of adrenaline.

2. Atropine reduced the responses to nerve stimulation, and appeared to have a more prominent effect on the responses elicited by stimulation at moderate frequency (10 pulses/sec).

3. Hexamethonium or nicotine caused a more powerful, but not complete, blockade of transmission ; the subsequent injection of atropine caused a further inhibition of the residual responses, leading to a complete, or near complete, abolition of the release by nerve stimulation.

4. The data were taken as evidence that transmission of impulses through muscarinic receptors occurs in normal conditions in the adrenal medulla of the dog, though this type of transmission is less prominent than that through nicotinic receptors.

Feldberg, Minz & Tsudzimura (1934) demonstrated in the cat that after the administration of large doses of nicotine, a minor residual release of catecholamines from the adrenal medulla occurred when the splanchnic nerve was stimulated. This response, which was potentiated by eserine and blocked by atropine, was considered by these investigators as evidence for the transmission of impulses from the splanchnic nerve endings to the chromaffin cells through muscarinic as well as nicotinic receptors. In a publication by Lee & Trendelenburg (1967) evidence was given that, under normal conditions, transmission of impulses in the adrenal medulla of the cat is mediated by nicotinic receptors only, but that transmission via a muscarinic mechanism is possible during the late phase of the blockade by large doses of nicotine. Hexamethonium has been shown to block almost completely the catecholamine release elicited by nerve stimulation from the adrenal medulla of the cat (Marley & Paton, 1961 ; Lee & Trendelenburg, 1967).

No reference was found concerning the nature of the transmission of impulses in the adrenal medulla of the dog. In a recent investigation (Kayaalp & Türker, 1969), it was demonstrated that the release of catecholamines from the adrenal medulla of

the dog due to intra-arterial injection of acetylcholine cannot be abolished by either ganglion blocking agents or atropine alone. This was confirmed in the main in a later work using a more precise fluorometric method for estimation of the catecholamines released (Kayaalp & McIsaac, 1969). In view of these observations an attempt was made in the present work to elucidate the nature of splanchnic-adrenal transmission in this species. Evidence will be presented that a minor component of transmission through muscarinic receptors is possible under normal conditions and also after blockade by hexamethonium as well as by nicotine.

Methods

Dogs of either sex, weighing between 9 and 15 kg, were anaesthetized with chloralose 100 mg/kg intravenously after induction with halothane. The trachea was cannulated for a patent airway. The left jugular vein was cannulated for intravenous injection of drugs. Both vagi were sectioned at mid-cervical level. The temperature of the animal was kept between 37·5° and 39° C by heating pads.

Stimulation of left splanchnic nerve

In order to reach the left splanchnic nerve at supradiaphragmatic level, the twelfth left rib was removed while the animal was lying on the right side, after starting artificial ventilation. The animal was kept in this position throughout the experiment. The splanchnic nerve was ligated and crushed as far proximal from the diaphragm as possible. A Harvard shielded stimulating electrode was placed on the peripheral portion of the nerve. The spread of the stimulating current was prevented by filling the thoracic cavity with paraffin oil sufficient to cover the nerve. In order to prevent the movement of the electrode by respiration, the diaphragm and intercostal muscles were paralysed by decamethonium (0·3 mg/kg intravenously). Half of this initial dose was repeated intermittently when required. Electrical stimulation was applied with a Grass stimulator delivering rectangular pulses of 0·5 msec duration and of supramaximal voltage through a stimulus isolation unit. The duration of stimulation was 1 min in all experiments.

Estimation of catecholamines released

The total amount of catecholamines released from the left adrenal gland into the circulation by stimulation of the left splanchnic nerve was estimated on the basis of the pressor responses in the acutely sympathectomized and perfused hindquarters of the same dog. The method was described in detail elsewhere (Kayaalp, 1968), so only a brief account will be given. The lumbar aorta was reached retroperitoneally through a longitudinal incision in the left flank. All collaterals of the aorta between the left renal artery and bifurcation were ligated. The hindquarters were denervated by cutting the lumbar sympathetic chain on both sides. The aorta was tied at mid-lumbar level and blood was circulated from the proximal portion of the aorta to the distal portion with an interposed tubing system equipped with a Sigmamotor pump. The coagulation of blood was prevented by heparin (600 u./kg intravenously). The mean perfusion pressure of the hindquarters was adjusted to lie between 60 and 80 mm Hg at the beginning of the experiment. The mean blood pressure was usually higher than 100 mm Hg. The blood pressure and perfusion pressure was recorded via Statham transducers from the side arms of the perfusion system, located proxi-

mal and distal to the pump respectively. The speed of the pump was kept constant throughout the experiment.

Since the sensitivity of the perfused vasculature to the catecholamines varies in different animals, the amount of catecholamine released by splanchnic nerve stimulation was estimated in terms of μg/kg adrenaline rather than in the magnitude of the increase in perfusion pressure. For this purpose adrenaline was injected intravenously in doses of 0·03, 0·1, 0·3, 1 and 3 or 4 μg/kg during the control period and after the administration of each blocking drug. The magnitude of the increase in perfusion pressure was plotted against the dose injected on semi-logarithmic paper. On the basis of the dose-response curve, the amount of adrenaline was found which would give the same magnitude of increase in perfusion pressure as was caused by stimulation of the splanchnic nerve. The reason for using changes in the perfusion pressure, rather than in the systemic blood pressure, to estimate catecholamine release is given in **Results**. As the pressor response elicited by nerve stimulation usually lasted longer than that elicited by single injection of adrenaline, the actual amount of catecholamine released by nerve stimulation was probably higher than that calculated on the basis of the technique used in this work. Since a comparison of the responses rather than their absolute amount was considered in the present work, however, the above-mentioned point was not a serious limitation.

Adrenal gland exclusion

In three experiments the contribution of the catecholamines released from the adrenal medulla to the pressor responses elicited by splanchnic stimulation was assessed after the exclusion of the left adrenal gland. Since a total adrenalectomy or the ligation of all vascular connections of the gland might interfere with the innervation of the abdominal sympathetic ganglia, a less traumatic technique was used for the exclusion. A loose ligature was placed around the left adrenolumbar vein between the vena cava and the gland after cannulating the same vein just lateral to the gland with polyethylene tubing. All branches of the adrenolumbar vein were tied.

The venous blood from the adrenal vein was allowed to flow out of the body through the cannula by gently pulling the loose ligature. Then stimulation of the left splanchnic nerve was started. The blood was kept flowing out until one minute after the end of stimulation. Thereafter the external end of the cannula was closed and the ligature was released so that the blood from the gland flowed into the vena cava. The significance of the difference between the means of the data obtained was calculated using Student's *t* test (Snedecor, 1956).

The following drugs were used (−)-adrenaline bitartrate, hexamethonium bromide, atropine sulphate, nicotine. All doses (with the exception of adrenaline and nicotine) refer to the salts.

Results

Effect of adrenal gland exclusion on the pressor responses

The influence of the exclusion of the left adrenal gland from the circulation as described in **Methods** was investigated on the pressor responses occurring in the systemic blood pressure and perfusion pressure upon splanchnic nerve stimulation. The results obtained with a stimulation frequency of 10 and 20 pulses/sec in three

experiments are summarized in Table 1 and the record of one of the experiments is shown in Fig. 1. After the exclusion of the adrenal gland the catecholamine equivalent released into the circulation due to splanchnic nerve stimulation was reduced to less than 5% of the control at both stimulation frequencies, whereas only a slight reduction was observed in the concomitant rise elicited in the systemic blood pressure. These data show that the pressor response in the perfusion pressure was due almost wholly to the substances released from the adrenal gland. The small residual response which was observed after exclusion of the gland might be due to a minor leakage of the adrenal venous blood into the circulation, though a minor rise in circulating catecholamines due to release from the postganglionic sympathetic nerve endings in the splanchnic area cannot be ruled out. On the other hand, the rise in systemic blood pressure was due not only to the release of catecholamines from the adrenal gland but also to a neurally mediated vasoconstriction in the splanchnic area.

The amount of catecholamines released by nerve stimulation was found to be less in these experiments than in animals with intact adrenal glands. This difference might arise from trauma to the gland while handling it for exclusion.

TABLE 1. *Contribution of catecholamines released from the adrenal gland to the pressor responses elicited by splanchnic nerve stimulation in three experiments*

Frequency of stimulation (pulses/sec)	Increase in perfusion pressure (mm Hg)		Increase in systemic blood pressure (mm Hg)	
	Intact	Adrenal gland excluded	Intact	Adrenal gland excluded
10	71·5±16·7 (0·88)	15·0±6·3 (0·03)	122·5±11·4	81·5±14·2
20	109·0±32·7 (2·54)	23·5±8·8 (0·05)	139·0±18·3	96·5±24·0

Values given are the means of the responses (±S.E.)
In parentheses are the equivalent amounts of catecholamine released by the nerve stimulation (as μg/kg adrenaline).

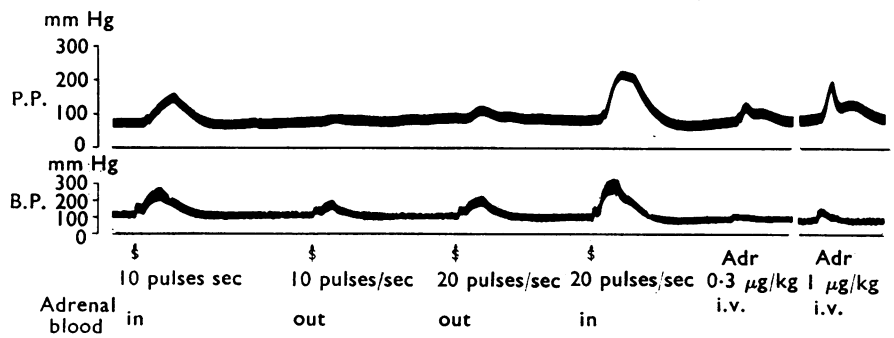

FIG. 1. Effect of exclusion of the left adrenal gland from the circulation on the pressor responses elicited by the stimulation of ipsilateral splanchnic nerve for 1 min at frequencies of 10 and 20 pulses/sec. Shown are the perfusion pressure (P.P.), systemic blood pressure (B.P.) and the pressor responses to the nerve stimulations ($\hat{\$}$) and to intravenous injections of adrenaline (Adr). The exclusion of adrenal gland was accomplished temporarily by allowing its venous blood to flow outside the body (see text). "Out" refers to the exclusion of the gland and "in" to normal venous flow inside the vena cava.

Effect of atropine on the responses of the adrenal gland to nerve stimulation

In nine experiments, the effect of atropine given intravenously in doses of 0·3–0·5 mg/kg (with the exception of one experiment in which 1 mg/kg was given) was studied before or after treatment with hexamethonium 10–12 mg/kg.

In five experiments of this series atropine was given after the control responses to stimulation of the left splanchnic nerve with 3, 10 and 20 pulses/sec. The stimulation of the splanchnic nerve with 1 pulse/second frequency did not cause any appreciable release of catecholamines, confirming the similar observation in the cat by Marley & Paton (1961), but significant release of catecholamines occurred at frequencies of 3, 10 and 20/sec. The stimulation was started at least 3 min after the injection of atropine. Atropine caused a reduction in the amount of catecholamine

TABLE 2. *Effect of atropine (0·3–0·4 mg/kg intravenously) and hexamethonium (10–12 mg/kg intravenously) on the catecholamine release from the left adrenal gland during stimulation of the ipsilateral splanchnic nerve at different stimulation frequencies applied for 1 min*

	Frequency of stimulus		
	3 pulses/sec	10 pulses/sec	20 pulses/sec
Control	0·16±0·04 (8)	1·78±0·32 (9)	3·78±0·47 (9)
After atropine	0·07±0·01 (5)	0·67±0·18* (5)	2·48±0·36 (5)
After subsequent hexamethonium	0·01±0·01* (5)	0·04±0·02* (5)	0·08±0·02* (5)

Values given are the equivalent of catecholamines released as μg/kg adrenaline (mean±S.E.). The number of experiments are shown in parentheses.
Asterisks indicate the values which are significantly different ($P<0·05$) from the upper one.

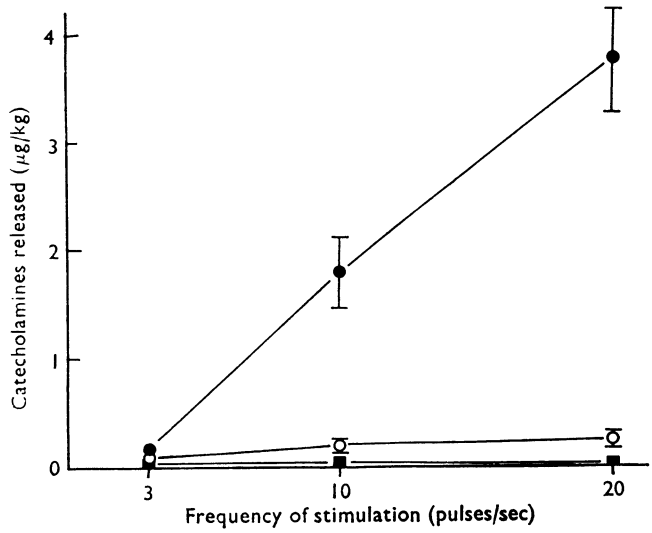

FIG. 2. Effect of atropine 0·3–1 mg/kg given intravenously after the intravenous injection of hexamethonium 10–12 mg/kg on the catecholamine release from the left adrenal gland elicited by stimulation of the ipsilateral splanchnic nerve for 1 min. The amount of catecholamine was expressed as the equivalent of adrenaline μg/kg. Shown are the control responses in untreated dogs (●——●; nine experiments, as in Table 2), the responses after the injection of hexamethonium (○——○) and atropine (■——■), each in four experiments. Note that all reductions in responses after either drug were statistically significant.

released from the adrenal medulla at each stimulation frequency (Table 2). The depression of the catecholamine release by atropine was greatest (59% of the control) for the stimulation with 10 pulses/sec ($P<0.02$). The depression of responses by atropine at the lower and upper frequency of stimulation was about 30% and not significantly different from the control ($0.05<P<0.10$).

The pressor responses elicited by nerve stimulation were also qualitatively different following atropine: although a steady rise in perfusion pressure was observed during nerve stimulation in the control period, the pressor response usually faded after an initial peak while the stimulation continued in atropine-treated dogs. The subsequent administration of hexamethonium caused a nearly complete abolition of the residual catecholamine release by nerve stimulation.

In four experiments of this series hexamethonium was injected before atropine and produced approximately 70–95% blockade of the stimulation-induced release of catecholamines (Fig. 2). Nevertheless, the subsequent injection of atropine still caused appreciable inhibition of the residual catecholamine release by nerve stimulation ($P<0.05$ for all frequencies of stimulation applied).

Effect of atropine on the response of adrenal medulla to nerve stimulation after treatment with nicotine

In three experiments the dogs were treated with nicotine given intravenously in three series of injections after the control responses were obtained. A period of 30–40 min was allowed to elapse between each series of injections. The first series

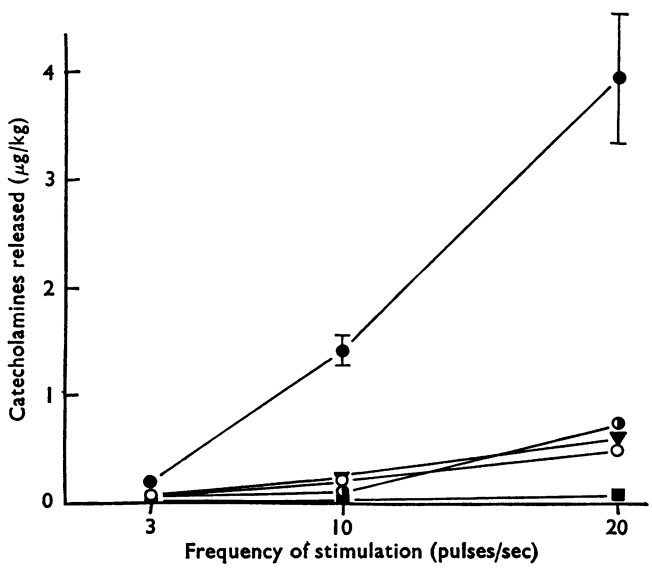

FIG. 3. Effect of atropine 0·3 mg/kg intravenously in three dogs treated with nicotine, given in three series of injections of 7, 10–12 and 10–12 mg/kg each in order of administration, on the catecholamine release from the left adrenal gland elicited by the stimulation of ipsilateral splanchnic nerve for 1 min. The amount of catecholamine released was expressed as the equivalent of adrenaline μg/kg. Shown are the control responses in untreated dogs (●——●), responses after first (◑——◑), second (▼——▼) and third (○——○) series of nicotine injections and responses after the subsequent injection of atropine (■——■).

of injections consisted of successive doses of 1, 3 and 3 mg/kg of nicotine and caused an 81–92% reduction of the amount of catecholamine released by nerve stimulation at different frequencies. The second and third series of nicotine injections, each consisting of two successive doses of nicotine 5–6 mg/kg, did not exert any remarkable change in the residual response to stimulation (Fig. 3). Nicotine itself caused a vigorous and sustained release of catecholamines when given at the beginning of the first series. The subsequent injections caused much less release of catecholamines. The adrenal medulla appeared to be completely unresponsive to all injections of the third series and the last injection of the second. The dose-response curve for adrenaline was not affected by nicotine.

Atropine (0·3 mg/kg intravenously) given approximately 25 min after the third series of nicotine injections caused a complete abolition of the response to 3 pulses/sec stimulation, and nearly complete abolition of the responses to stimulations with higher frequencies ($P<0·05$). Nicotine (5 mg/kg intravenously) caused no release of catecholamines when it was given at the end of the experiment.

Discussion

In previous studies (Kayaalp & Türker, 1969 ; Kayaalp & McIsaac, 1969), it was found that the release of catecholamines from the adrenal medulla into the venous effluent by acetylcholine given intra-arterially at an optimal dose level was quite resistant to blockade by hexamethonium and mecamylamine. However, the remarkable inhibition by hexamethonium of the catecholamine release due to splanchnic nerve stimulation points to a possible dissociation between the effect of this drug on the release by nerve stimulation and exogenous acetylcholine. These observations seem to be in line with those by Feldberg *et al.* (1934) in the cat. These investigators did not observe any substantial change after nicotine in the catecholamine release following intra-arterial acetylcholine (see their Fig. 9), though a comparable dose of nicotine caused a nearly complete blockade of the release caused by splanchnic nerve stimulation in other experiments. This dissociation may be explained on the basis of the assumption that the localization of nicotinic and muscarinic receptors is heterogeneous in the chromaffin cell membrane, so that nicotine receptors are mostly located in the postsynaptic patch and thus more available for activation upon nerve stimulation, whereas muscarinic receptors may be mostly located outside the synaptic area.

A reduction of the resting release of catecholamines from the adrenal medulla was observed by Malmejac, Chardon & Gross (1953) following the injection of atropine in the dog. But this action was reported to be of very short duration (a few minutes). In the present work atropine inhibited catecholamine release by splanchnic nerve stimulation with frequencies ranging from 3 to 20 pulse/sec. However, inhibition of only the response to 10 pulses/sec was statistically significant. It is highly unlikely that the inhibition was due to a hexamethonium-like effect of atropine, because atropine (1 mg/kg intravenously) did not affect the catecholamine release by dimethylphenylpiperazinium, though it blocked completely the release by methacholine (Kayaalp & Türker, 1969).

The release by nerve stimulation after the partial blockade of transmission by either hexamethonium or nicotine appeared to be more susceptible to the inhibitory action of atropine, in that the blockade by atropine was relatively more prominent and significant for all the frequencies of stimulation used. The combination of

atropine and hexamethonium or nicotine produced a complete or near to complete blockade of the release by nerve stimulation. In the cat, atropine was shown to block only the residual responses of the adrenal medulla after the administration of large doses of nicotine (Feldberg *et al.*, 1934; Lee & Trendelenburg, 1967), but they did not observe any appreciable blockade of the responses after the administration of atropine alone. Evidence was recently given to support the involvement of muscarinic receptors, in addition to nicotinic ones, in transmission through the autonomic ganglia of the dog (Flacke & Gillis, 1968). In their experiments, the response of the heart to preganglionic sympathetic stimulation was not abolished, but only shifted to a higher frequency following the administration of ganglion blocking agents, and the residual response was completely abolished by a small dose of atropine. Since the autonomic ganglia are the developmental analogues of the adrenal medulla, these observations seem to agree with the results of the present work. These investigators, however, did not observe any inhibition of transmission when atropine was given alone.

In conclusion, the results of the present study show that the transmission of impulses from the splanchnic nerve endings to the chromaffin cells may occur partly through muscarinic receptors in normal conditions in the adrenal medulla of the dog, unlike that of the cat. The comparison of the magnitude of the blockade by atropine on the one hand, and by hexamethonium or nicotine on the other, implies that the involvement of muscarinic receptors in the transmission may be less important than that of nicotinic receptors. A complete blockade of transmission may be obtained by the administration of a ganglion blocking agent in combination with atropine. Either drug, when given alone, causes only a partial inhibition of transmission.

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REFERENCES

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

FLACKE, W. & GILLIS, R. A. (1968). Impulse transmission via nicotinic and muscarinic pathways in the stellate ganglion of the dog. *J. Pharmac. exp. Ther.*, **163**, 266–276.

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

KAYAALP, S. O. & MCISAAC, R. J. (1969). *In vivo* release of catecholamines from the adrenal medulla by selective activation of cholinergic receptors. *Archs int. Pharmacodyn. Thér.*, **176**, 168–175.

KAYAALP, S. O. & TÜRKER, R. K. (1969). Evidence for muscarinic receptors in the adrenal medulla of the dog. *Br. J. Pharmac.*, **35**, 265–270.

LEE, F. L. & TRENDELENBURG, U. (1967). Muscarinic transmission of preganglionic impulses to the adrenal medulla of the cat. *J. Pharmac. exp. Ther.*, **158**, 73–79.

MALMEJAC, J., CHARDON, G. & GROSS, A. (1953). Atropine et adrénalino-secrétion. *C. r. Séanc. Soc. Biol.*, **147**, 798–801.

MARLEY, E. & PATON, W. D. M. (1961). The output of sympathetic amines from the cat's adrenal gland in response to splanchnic nerve activity. *J. Physiol., Lond.*, **155**, 1–27.

SNEDECOR, G. W. (1956). *Statistical Methods.* Ames, Iowa: Iowa State Univ. Press.

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