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POTENTIATION OF INHIBITORY AND EXCITATORY EFFECTS OF CATECHOL AMINES BY BRETYLIUM

BY

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Enhancement of both excitatory and inhibitory responses to adrenaline, noradrenaline and isoprenaline was demonstrated with bretylium both *in vivo* and *in vitro*. Excitatory responses studied included rise of blood pressure and contraction of the nictitating membrane in cats and the contraction of aortic strip in rabbits; the inhibitory responses studied were fall of cat blood pressure and relaxation of rat uterus and rabbit tracheal chain. Possible mechanisms of potentiation of the effects of catechol amines by bretylium are discussed. A sensitization of the peripheral effector cells is suggested as a likely mechanism of potentiation.

A new hypotensive drug, bretylium, has recently been introduced in therapeutics. Pharmacological tests on animals have shown that it blocks adrenergic neurones, without antagonizing the effects of released or injected adrenaline or noradrenaline (Boura & Green, 1959). Studying the effects of bretylium on cardiovascular control in dogs, Aviado & Dil (1960) reported that after the injection of bretylium the pressor response induced by noradrenaline was enhanced and prolonged. Vernikos-Danellis & Zaimis (1960) have also reported a potentiation of the blood pressure effects of adrenaline and noradrenaline after pretreatment with bretylium. The final explanation for the augmentation of the effects of these drugs by bretylium is still not certain. One possibility is that the effector cells in heart and blood vessels are sensitized to adrenaline and noradrenaline. Another possibility is that bretylium blocks the compensatory reflexes that are known to be excited by any pressor agent. If bretylium is shown to have a peripheral action in the form of a sensitization of the effector cells to adrenaline and noradrenaline, it might help to explain the " tolerance " that is so often reported with bretylium in clinical practice.

An attempt is made to characterize the potentiation of the blood pressure effect of adrenaline, noradrenaline and isoprenaline by bretylium. The effect of bretylium on other excitatory and inhibitory responses to adrenaline, noradrenaline and isoprenaline is also studied.

METHODS

Cats. The effect of bretylium on blood pressure and nictitating membrane responses to noradrenaline, adrenaline and isoprenaline was studied in intact and in vagotomized cats anaesthetized with chloralose 80 mg/kg and in spinal vagotomized cats. Blood pressure was recorded from a cannula in the left common carotid artery. The nictitating membrane of

the right side was connected to a frontal writing lever and isotonic contractions were recorded on a smoked paper. All drugs were dissolved in normal saline and injected through a polyethylene cannula in the femoral vein.

Fixed doses of noradrenaline, adrenaline or isoprenaline were administered rapidly at 10 min intervals. When the response produced by a fixed dose was reproducible, bretylium was administered in a dose of 5 mg/kg; the catechol amine was again administered 15 min after injection of bretylium and at 10 min intervals for 1 hr. The following measurements were made of the responses to adrenaline, noradrenaline and isoprenaline: △ B.P.—the difference between the control systolic blood pressure and peak systolic pressure of the pressor or depressor response expressed in mm of Hg; decay time 50 (DT 50): the time in sec from the onset of the pressor or depressor response to the time when the response had decreased to one-half of the maximum.

Aortic strips. Strips were obtained from the thoracic aorta of young rabbits and prepared in the manner described by Furchgott & Bhadrakom (1953). Spirally cut aortic strips approximately 3.5 cm in length were placed in a 50 ml. organ bath and tied to an isotonic frontal writing lever that placed the muscle under 5 g of tension at the horizontal equilibrium position. The bathing solution was Krebs bicarbonate solution containing 0.01 M glucose. The solution was maintained at 37 to 38° C, and 5% carbon dioxide in oxygen was bubbled through the solution in both bath and reservoir. The preparation was left for 2 hr before testing.

Dose-response curves for noradrenaline and adrenaline were obtained by cumulative administration of increasing concentrations of the amines, allowing the contraction to develop fully after each administration. Dose-response relationships for noradrenaline and adrenaline were determined in replicate on each strip prior to administration of bretylium. In studies on potentiation, bretylium was placed in the bath 5 min before administration of catechol amines and remained in the bath thereafter.

Tracheal chains. Rabbit tracheal chains were prepared according to the method of Castillo & deBeer (1947). Preparations were suspended in a 50 ml. organ bath and connected to a balsa-wood frontal writing lever. Krebs bicarbonate solution containing 0.01 M glucose and aerated with 95% oxygen and 5% carbon dioxide was used as the bathing medium. Pilocarpine was added in a concentration of 1 mg/100 ml. The responses to adrenaline and isoprenaline were recorded on smoked kymograph paper after the pilocarpine-induced spasm had reached a stable plateau.

Rat uterus. Uterine horns of non-pregnant rats were suspended in a 30 ml. bath and connected to a frontal writing lever. De Jalon fluid aerated with 95% oxygen and 5% carbon dioxide and maintained at 30° C was used as the bathing medium. Reduction of acetylcholine-induced contractions of the rat uterus was taken as a measure of the inhibitory effect of adrenaline and isoprenaline. When the inhibition produced by a fixed dose was constant, bretylium was added to the bath and allowed to act for 30 sec; the inhibitory effect of the amines was again determined.

Drugs. Bretylium was used as bretylium tosylate, and the doses or concentrations refer to the salt. Adrenaline and noradrenaline were used as the base dissolved in normal saline and the doses or concentrations refer to the base. Isoprenaline was used as isoprenaline sulphate and the doses or concentrations refer to the salt.

RESULTS

Effect of bretylium on cat blood pressure and nictitating membrane responses to catechol amines. Moderate doses of bretylium (5 to 7 mg/kg) administered rapidly intravenously always produced a biphasic response in blood pressure in all the preparations. There was a brief initial fall followed by a rise which persisted for

TABLE 1

EFFECT OF BRETYLIUM (5 MG/KG) ON MAGNITUDE AND DURATION OF BLOOD PRESSURE RESPONSES TO ADRENALINE, NORADRENALINE AND ISOPRENALINE

$$\% = \frac{\text{Value in treated animal}}{\text{Value in untreated animal}} \times 100$$

	Adrenaline (0·5 to 2·0 μg/kg)			Noradrenaline (0·5 to 2·0 μg/kg)			Isoprenaline (0·5 to 2·0 μg/kg)		
	Type of preparation								
	Intact	Vagotomized	Spinal vagotomized	Intact	Vagotomized	Spinal vagotomized	Intact	Vagotomized	Spinal vagotomized
Untreated Δ B.P. mm of Hg	30	77	42	38	64	47	16	77	20
Treated Δ B.P. mm of Hg	48	101	98	73	90	126	27	111	36
%	160	130	230	190	140	260	170	140	190
	($P<0·01$)	($P<0·05$)	($P<0·01$)	($P<0·01$)	($P<0·01$)	($P<0·01$)	($P<0·01$)	($P<0·01$)	($P<0·05$)
Untreated DT 50 sec	69	120	90	60	97	72	34	36	66
Treated DT 50 sec	114	196	116	66	178	97	48	51	95
%	165	163	129	110	183	134	141	141	144
	($P<0·05$)	($P<0·01$)	($P>0·05$)	($P<0·01$)	($P<0·01$)	($P<0·01$)	($P<0·01$)	($P<0·01$)	($P<0·01$)

10 to 12 min. During the later portion and the peak of the pressor response there was an increase in pulse pressure. As a rule blood pressure stabilized at or slightly above control blood pressure within 15 min.

Table 1 summarizes the changes produced by bretylium in the vasomotor reactivity of intact, vagotomized and spinal vagotomized cats to fixed doses of adrenaline, noradrenaline and isoprenaline (0.5 to 2.0 μg/kg). The control values are means of 3 to 8 observations made in 2 or 3 animals, while the values after treatment with bretylium represent the mean of 8 to 14 observations made at 10 min intervals in 3 to 4 cats for 1 hr following the administration of bretylium.

Doses of 5 to 7 mg/kg of bretylium produced a simultaneous potentiation of the pressor effect and of the response of the nictitating membrane to adrenaline and noradrenaline in intact, vagotomized and spinal vagotomized cats. It also potentiated the depressor effect of isoprenaline (Figs. 1 and 2).

The potentiating effect of bretylium on the blood pressure responses to the catechol amines was best seen in the spinal vagotomized preparation, the magnitude of the responses to adrenaline, noradrenaline and isoprenaline being enhanced to 230, 260 and 190% respectively of the control values. Bretylium also prolonged the mean duration of the pressor or depressor response to the catechol amines. The effect was more significant for noradrenaline and isoprenaline than for adrenaline.

Aortic strips. The major difficulty of *in vitro* investigation of the action of sympathomimetic amines has been the selection of a suitable biological test preparation. Furchgott (1955) has shown that the aortic strip preparation fulfils many of

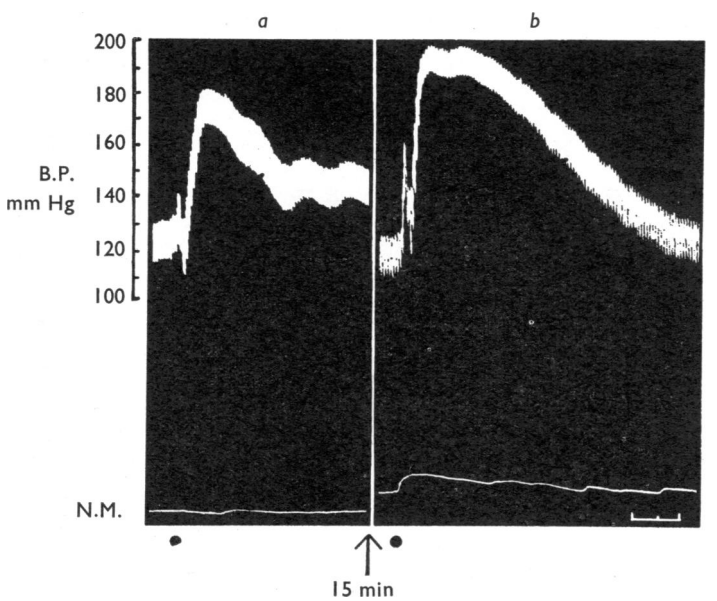

Fig. 1. Cat, 3 kg, chloralose anaesthesia. Both vagi cut in the midcervical region. Records of carotid arterial blood pressure (B.P.) and nictitating membrane (N.M.). Responses to intravenous injections of 6 μg of noradrenaline (at the dots) before (*a*) and 15 min after 5 mg/kg bretylium (*b*). Time, 1 min.

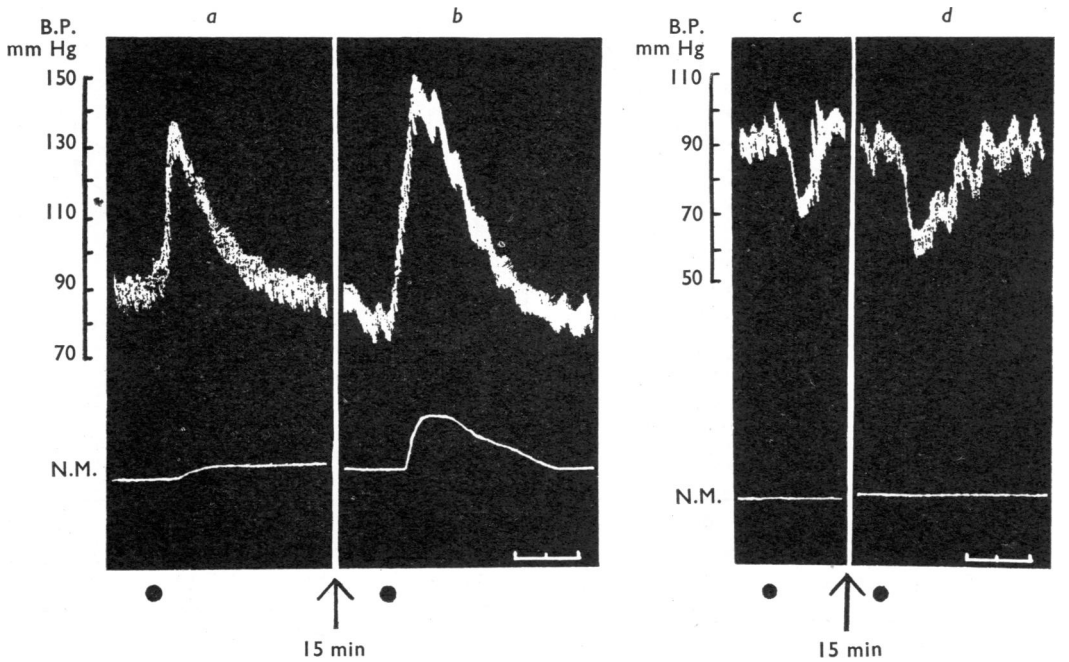

Fig. 2. Cat, 2.6 kg, spinal vagotomized preparation. Records of carotid arterial blood pressure (B.P.) and nictitating membrane (N.M.). Responses to intravenous injections (at the dots) of 5 μg adrenaline (*a* and *b*) and of 5 μg isoprenaline (*c* and *d*) before (*a* and *c*) and 15 min after (*b* and *d*) 5 mg/kg bretylium. Time, 1 min.

the requirements of an ideal test object. It contains only one type of pharmacologically active cell, and after an initial equilibration in saline it shows no residual tone and no spontaneous activity. This preparation was therefore chosen to study the effect of bretylium on the excitatory responses of adrenaline and noradrenaline *in vitro*.

A concentration of 2×10^{-5} of bretylium potentiated the contractions induced by adrenaline and noradrenaline (Table 2). In Fig. 3 the responses to adrenaline and

TABLE 2

ENHANCEMENT BY BRETYLIUM OF EXCITATORY AND INHIBITORY RESPONSES TO CATECHOL AMINES

— indicates no detectable response

Preparation	No. of experiments	Dose or concentration of bretylium	Average % control response		
			Noradrenaline	Adrenaline	Isoprenaline
Nictitating membrane	5	5 mg/kg	250	210	—
Aortic strips	6	2×10^{-5}	155	116	—
Rat uterus	6	2×10^{-6}	—	144	—
	6	5×10^{-6}	—	165	—
	6	1×10^{-5}	—	177	134
	7	2×10^{-5}	—	221	154
Tracheal chain	6	5×10^{-6}	—	183	120
	6	1×10^{-5}	—	300	135
	6	2×10^{-5}	—	500	150

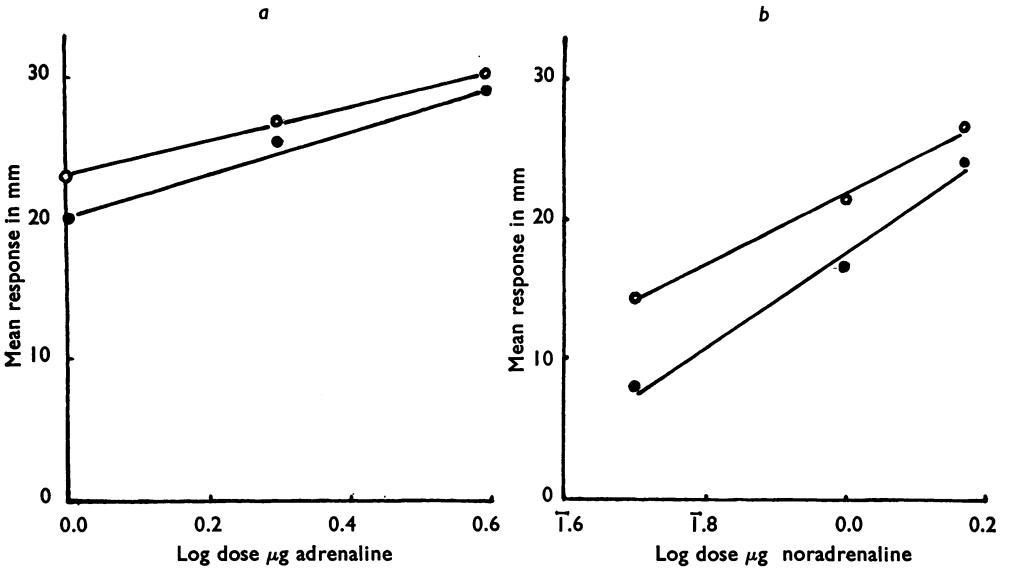

Fig. 3. Log dose-response curves of (*a*) adrenaline and (*b*) noradrenaline on isolated rabbit aortic strips, alone (closed circles) and in the presence of bretylium (open circles).

noradrenaline are plotted on a graph to give the dose-response curves, alone and in the presence of bretylium. The potentiation is more pronounced with lower doses of the amines and decreases with higher doses.

Tracheal chain. The rabbit tracheal chain was used to study the effect of bretylium on inhibitory responses to adrenaline and isoprenaline. The responses of the preparation to adrenaline and isoprenaline varied with repeated doses ; the addition of pilocarpine to the bathing fluid enabled a uniform inhibitory response to be obtained.

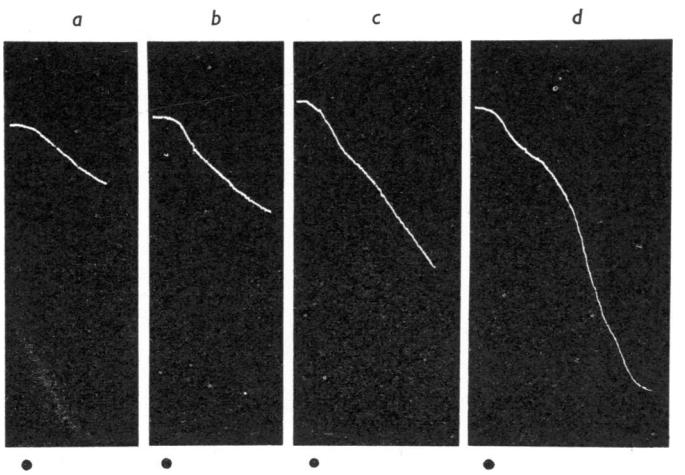

Fig. 4. Responses of the rabbit tracheal chain to adrenaline 1×10^{-6} (at the dots) alone (*a*) and in the presence of bretylium 5×10^{-6} (*b*), bretylium 1×10^{-5} (*c*), and bretylium 2×10^{-5} (*d*). Contact time, 2 min.

Bretylium in concentrations of 5×10^{-6}, 1×10^{-5} and 2×10^{-5} did not alter the pilocarpine-induced spasm, but after exposure for 1 min the relaxing effect of adrenaline or isoprenaline was considerably enhanced (Table 2). Fig. 4 shows the potentiation of the relaxant effect of adrenaline (1×10^{-6}) by increasing concentrations of bretylium. The potentiation was related to the dose.

Rat uterus. The most sensitive biological method of estimating adrenaline is by its inhibition of acetylcholine-induced contractions of the rat uterus. The percentage reduction was determined before and in the presence of various concentrations of bretylium. Bretylium concentrations of 2×10^{-5}, 1×10^{-5}, 5×10^{-6} and 2×10^{-6} greatly enhanced the inhibitory action of adrenaline or isoprenaline (0.6×10^{-9}) on the rat uterus (Table 2). Fig. 5 shows the inhibitory action of

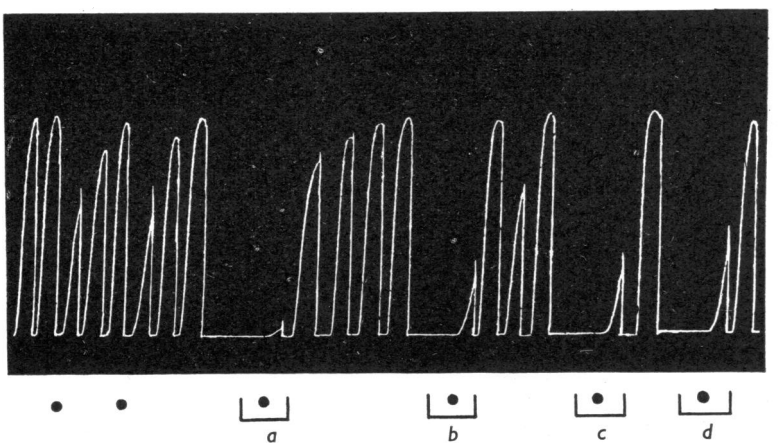

Fig. 5. Inhibitory action of adrenaline 0.6×10^{-9} (added at the dots) on acetylcholine (0.3×10^{-6}) induced contractions of the rat uterus. Contact time 30 sec for both acetylcholine and adrenaline. The period marked by bar (*a*) indicates the presence of bretylium 2×10^{-5}, (*b*) bretylium 1×10^{-5}, (*c*) bretylium 5×10^{-6}, and (*d*) bretylium 2×10^{-6}.

0.6×10^{-9} of adrenaline before and in the presence of varying amounts of bretylium. The potentiation produced by bretylium is related to the dose. Bretylium in the highest concentration used, that is, 2×10^{-5}, did not affect acetylcholine-induced contractions.

DISCUSSION

The present study has shown that bretylium enhances *in vivo* the magnitude and duration of certain excitatory and inhibitory effects of the three catechol amines employed, adrenaline, noradrenaline and isoprenaline. This potentiation could not be ascribed to the suppression by bretylium of the baroreceptive reflexes, since potentiation is obtainable in bilaterally vagotomized animals and spinal vagotomized cats. Further, Aviado & Dil (1960) have reported that bretylium alone could not block the increase of blood pressure due to the carotid sinus reflex elicited by bilateral clamping of the common carotid arteries. A combination of adrenalectomy and bretylium was required to abolish the carotid sinus reflex. Moreover, the dose of bretylium required (20 mg/kg) was far too high. Thus a blockade of the

compensatory cardiovascular reflexes by bretylium can be eliminated as a cause of potentiation.

From studies of the effects of sympathomimetic amines in normal and reserpine pretreated animals, Burn & Rand (1958) have proposed that ephedrine and other non-catechol compounds of this class act primarily by releasing a noradrenaline-like substance from available stores in the vascular walls. In terms of this concept the heightened response to catechol amines immediately following a dose of ephedrine might be attributed to temporary depletion of the stores and a consequent availability of a larger proportion of the receptor sites in the vascular smooth muscle to injected catechol amines. A similar mechanism for the action of bretylium in potentiating the effects of catechol amines is not excluded by our results.

Mantegazza, Tyler & Zaimis (1958) have reported that the ganglion-blocking agents, hexamethonium and pentolinium, enhance the responses of the nictitating membrane and peripheral blood vessels to injected catechol amines. This action was attributed to sensitization of the effector cells, possibly through changes in permeability. A similar explanation could be suggested for the potentiation of the catechol amines by bretylium. We have been able to demonstrate enhancement of the action of the catechol amines *in vitro* by bretylium. This potentiation of the *in vitro* effects of the catecholamines by bretylium indicates sensitization of the effector cells as the mechanism of potentiation.

Thus sensitization of the effector cells in the heart and blood vessels may well account for the increase in pressor response produced by adrenaline or noradrenaline in presence of bretylium, and for the development of tolerance which appears during the treatment of hypertensive patients.

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