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A quantitative analysis of the antagonism of intravenous noradrenaline by thymoxamine or phentolamine on the blood pressure of the conscious cat

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The blood pressure of the unanaesthetized cat was recorded from a nylon catheter inserted permanently into the aorta via the right carotid artery and intravenous injections were made into a nylon catheter tied permanently into the right external jugular vein. For noradrenaline there was a linear relation between log dose and blood pressure rise. The α-blocking agents thymoxamine and phentolamine lowered the blood pressure and decreased the pressor response to noradrenaline. Quantitative analysis of the results by three different graphical methods revealed that for the first dose of blocking agent the antagonism was complex but had the characteristics associated with mixed non-competitive and uncompetitive antagonism. For further cumulative doses of blocking agent, the antagonism had the characteristics of competitive antagonism. It is concluded that a first dose of α-blocking agent has an effect on the dynamics of a noradrenaline-induced blood pressure rise in the conscious cat which is complex and could in part be due to the initial effect of the blocking agent in lowering the blood pressure and in part to the activity of the vasomotor compensatory reflexes. After α-blockade has been initiated, the characteristics of the antagonism are, like those in the anaesthetized cat, those of competitive antagonism.

QUANTITATIVE analysis of the effects of adrenergic blocking agents on the responses of the cardiovascular system to injected catecholamines is likely to be more complex than that for adrenergic blockade of vascular tissue *in vitro*, but Chen & Russell (1950) and Matsumoto & Kumoi (1958) showed, for anaesthetized animals, that by suitable graphical treatment of the results such analysis was possible. Thuránszky (1966a) established that there are qualitative differences between anaesthetized and unanaesthetized cats in the responses of the blood pressure to adrenaline and to adrenergic blocking agents.

This paper describes a quantitative analysis of the blood pressure responses of unanaesthetized cats to intravenous noradrenaline alone and to noradrenaline in the presence of adrenergic blocking agents. For one group of cats the α-blocking agent was thymoxamine, 4-(2-dimethylaminoethyl)-5-isopropyl-2-methylphenyl acetate, which was shown by Birmingham & Szolcsányi (1965) to be a competitive antagonist of noradrenaline on isolated arterial strips and by Greef & Schümann (1953) to reduce or abolish in anaesthetized cats, the rise in blood pressure associated with the intravenous injection of noradrenaline. For a second group of cats the α-blocking agent was phentolamine (Meier & Yonkman, 1949; Walker, Heymans, Wilson & Richardson, 1950).

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Experimental

METHODS OF CANNULATION

Blood pressure was recorded from a catheter placed permanently in the aorta and drugs were injected through a catheter tied permanently into the external jugular vein. The method of cannulation and daily management of the cats was based on that developed by Thuránszky (1966b). Cats of either sex weighing from 1·75 to 3·5 kg were anaesthetized with ether. The right external jugular vein was exposed through an incision to the right of the midline and cannulated with Portex No. 3 standard nylon tubing of outside diameter 1·25 mm and bore 0·75 mm. Some 3 cm of tubing was inserted into the vein and about 7 cm protruded. The external end was pushed into closely fitting soft rubber tubing, about 3 cm in length, and sealed with a glass stopper after filling with saline. The catheter was tied firmly into the vein by three ligatures. Next, the right carotid artery was prepared for cannulation and at this stage 0·1 ml of 5,000 IU/ml heparin was injected into the venous cannula. The arterial cannula (also Portex No. 3) was approximately the same length as the distance from the xiphoid process to the tip of the mandible. The catheter was connected by a soft rubber tube to a syringe, filled with saline and inserted into the carotid artery for about 2 cm and held in place by one loosely-tied ligature. The syringe was now replaced by a polythene tube leading to a Statham pressure transducer, and while the blood pressure was recorded, the arterial catheter was advanced into the aorta until about 8 cm remained. At this point, with the tip of the catheter in the descending thoracic or upper abdominal aorta, two firm ligatures were tied around the catheter in the carotid artery. The catheter was then filled with heparin and closed with a glass stopper. The incision was closed with Michel clips leaving both catheters protruding from the top end. The incision and the catheters were protected with a cotton bandage. Although the cats made a quick recovery from the operation they were not used in an experiment until the fourth day because Thuránszky, Rablóczky & Kékes-Szabó (1966) showed that the responses to injected catecholamines were altered by anaesthesia and this alteration persisted, in varying degrees, for 72 hr after the recovery of consciousness.

EXPERIMENTS

During an experiment each cat was housed in a large cage within which it could move freely. The bandage was removed and the venous cannula was connected to a long polythene tube through which drug solutions were injected. The dead space of the system was 1·4 ml. The arterial cannula was similarly connected to a Statham pressure transducer for recording blood pressure on a Grass 7 Polygraph. Both polythene tubes were elastically-suspended above and through the roof of the cage so that the movements of the cat were not impeded. The experiment was not begun until the cat was lying at rest and the blood pressure had stabilized. After ensuring that there was no change in blood pressure to injections of

normal saline, the responses to intravenous noradrenaline were recorded. Doses of 0·1, 0·2, 0·4 and 0·8 μg/kg noradrenaline were injected with an interval of 4 to 5 min between doses. Then the first dose of adrenergic blocking agent, thymoxamine or phentolamine, was injected. When the blood pressure had stabilized again the same four doses of noradrenaline were repeated. This procedure was repeated until a total of three doses of adrenergic blocking agent had been given. The cats showed no signs of disturbance from the changes in blood pressure induced by the drugs. Different cats were used for thymoxamine and for phentolamine. For the few cats used more than once, at least 3 days were allowed to elapse between experiments.

DRUGS

These were: noradrenaline bitartrate, phentolamine hydrochloride and thymoxamine hydrochloride; they were dissolved in normal saline and doses refer to the base/kg bodyweight.

Results

ANTAGONISM OF NORADRENALINE BY THYMOXAMINE OR PHENTOLAMINE

Table 1 shows the mean results for thymoxamine obtained from six experiments made on five cats. Before thymoxamine, the noradrenaline-induced rise in blood pressure increased with each increase in dose (x) of noradrenaline, the increase in blood pressure (y) ranged from 30 mm Hg for 0·1 μg/kg noradrenaline to 90 mm Hg for 0·8 μg/kg. After treatment with 4 mg/kg thymoxamine, the same doses of noradrenaline produced much smaller rises in blood pressure (y′). Two more increments of 4 mg/kg thymoxamine produced further but smaller reductions in the responses to the four doses of noradrenaline. Also shown in Table 1 are the mean results for phentolamine from seven experiments on another five cats.

TABLE 1. MEANS OF THE PRESSOR RESPONSES TO INTRAVENOUS NORADRENALINE ALONE AND IN THE PRESENCE OF THYMOXAMINE OR PHENTOLAMINE (the standard errors are shown in parentheses)

x, the dose of noradrenaline (μg/kg)	y, the pressor response to noradrenaline (mm Hg)	y′, the pressor response to noradrenaline (mm Hg) after each cumulative dose of antagonist		
		Thymoxamine mg/kg		
		4	8	12
0·1	30·2 (3·7)	20·7 (2·3)	16·0 (3·5)	14·5 (5·3)
0·2	52·8 (4·5)	34·2 (4·4)	25·8 (2·9)	24·8 (5·1)
0·4	72·0 (8·0)	45·3 (5·1)	44·3 (8·1)	37·5 (7·8)
0·8	90·5 (9·8)	55·5 (5·3)	54·5 (8·1)	49·0 (9·6)
		Phentolamine mg/kg		
		0·2	0·4	0·6
0·1	18·6 (2·1)	12·4 (1·3)	10·0 (2·2)	6·0 (4·0)
0·2	38·1 (4·5)	25·0 (5·2)	16·9 (4·4)	16·2 (5·4)
0·4	56·1 (5·0)	37·8 (7·6)	33·4 (5·0)	32·0 (7·5)
0·8	81·3 (9·6)	51·6 (7·5)	50·9 (6·4)	42·7 (8·1)

The results from Table 1 are shown as log dose response graphs, with regression lines, in Fig. 1. The logarithm of the noradrenaline dose

(log x) was plotted as abscissa against the rise in blood pressure as ordinate for noradrenaline alone (y) and for noradrenaline after the first, second and third dose of antagonist (y'). For noradrenaline alone there was a linear relation between log dose and response. After 4 mg/kg thymoxamine or 0·2 mg/kg phentolamine the relation was still linear but the response to noradrenaline was reduced. The reduction was greater the higher the noradrenaline dose so that the regression line had a decreased slope. After each subsequent dose of 4 mg/kg thymoxamine or 0·2 mg/kg phentolamine there was a further decrease in the response to noradrenaline. The reductions were less pronounced than that produced by the first dose of antagonist and there appeared to be no further change in the slopes of the regression lines.

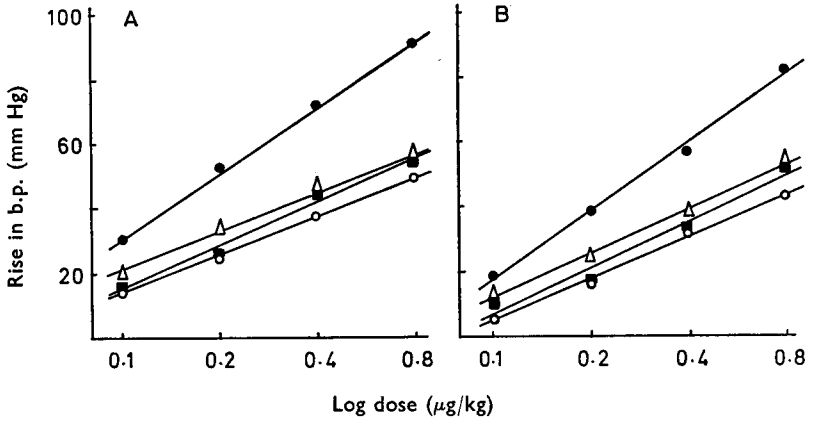

FIG. 1. Log dose-response graphs of the effects of thymoxamine (A) or of phentolamine (B) on the rise in blood pressure induced by intravenous noradrenaline in the conscious cat. The ordinates show the increase in blood pressure in mm Hg (the blood pressure before the injection subtracted from the blood pressure at the height of the response to the noradrenaline injection). The abscissae show the doses of noradrenaline in μg/kg plotted on a logarithmic scale. A. Each point is a mean of six observations on five cats: ●——● the response to noradrenaline alone, △——△, ■——■ and ○——○ the responses to noradrenaline after cumulative total doses of 4, 8, and 12 mg/kg thymoxamine respectively. B. Each point is a mean of seven observations on five cats: ●——● the response to noradrenaline alone, △——△, ■——■ and ○——○ the responses to noradrenaline after cumulative total doses of 0·2, 0·4 and 0·6 mg/kg phentolamine respectively.

In Fig. 2 the results from Table 1 are shown plotted as reciprocals of noradrenaline dose (abscissa), 1/x, against reciprocals of rise in blood pressure (ordinate) for noradrenaline alone, 1/y, and for noradrenaline in the presence of the antagonists, 1/y'. Calculated regression lines were fitted to each set of mean results. For noradrenaline alone there was a linear relation between 1/x and 1/y. After 4 mg/kg thymoxamine or 0·2 mg/kg phentolamine there was still a linear relation between 1/x and 1/y' but the lines had steeper slopes and different intercepts with the ordinates when compared with the control lines (1/x against 1/y). The subsequent doses of thymoxamine or phentolamine produced 1/x against

1/y′ plots each with an increased slope but all the lines in the presence of the antagonists had a common intercept on the ordinate. When the total dose of phentolamine had reached 0·6 mg/kg, the straight line relation appeared to break down at the lowest dose of noradrenaline as shown by the sharp increase in slope of the line joining the calculated line for the three higher doses to the point for the lowest dose of noradrenaline.

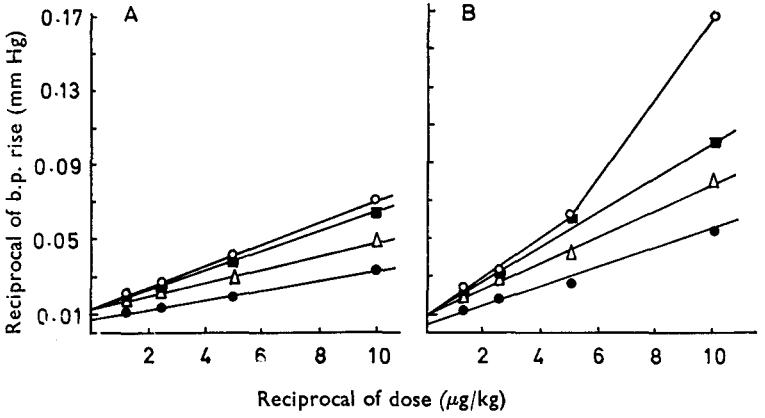

FIG. 2. Lineweaver and Burk plots of the results shown in Fig. 1. A. Thymoxamine. B. Phentolamine. Ordinates: the reciprocal of the increase in blood pressure in mm Hg. Abscissae: the reciprocal of the dose of noradrenaline in μg/kg injected intravenously. Key to symbols as in Fig. 1.

Further analysis of the antagonism of noradrenaline was made by plotting the logarithm of the dose of noradrenaline (log x) as abscissa against the logarithm of the rise in blood pressure (y or y′) divided by the difference between the rise in blood pressure and the maximum rise in blood pressure (y_{max} or y'_{max}) as ordinate (Fig. 3). The maximum rise in blood pressure was not measured directly but was calculated (Chen & Russell, 1950) from the point of intercept of the dose response line with the ordinate in Fig. 2. This analysis was made only for noradrenaline alone and for the first dose of phentolamine or thymoxamine. Regression lines were fitted to the points and Fig. 3 shows that there was a linear relation for log x against log $[y/(y_{max} - y)]$ or log $[y'/(y'_{max} - y')]$ when plotted by this method and that the lines for response to noradrenaline after one dose of antagonist were above and parallel to the lines for noradrenaline alone ($P > 0{\cdot}9$ for phentolamine; $P = 0{\cdot}8$ to $0{\cdot}9$ for thymoxamine).

THE EFFECTS OF THYMOXAMINE OR PHENTOLAMINE ON THE BLOOD PRESSURE

When thymoxamine (4 mg/kg) was injected there was usually a sudden and profound fall in blood pressure which lasted for 30–40 sec (approximately the period of injection) which was then replaced by a short-lived rise before the blood pressure stabilized at a level below that of the pre-injection pressure. There was no comparable sudden fall in blood

pressure with phentolamine, the injections of 0·2 mg/kg were usually followed by a slow and gradual fall in blood pressure to a new stable level below that of the pre-injection pressure. From the records made in the experiments on antagonism of noradrenaline, the effects of thymoxamine or phentolamine on the blood pressure of the conscious cat were measured as the difference between the stable pre-injection blood pressure

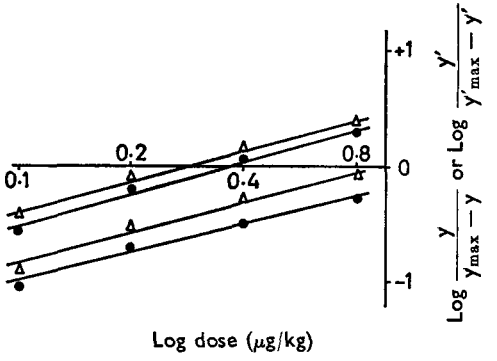

FIG. 3. The effects of the first dose of thymoxamine or phentolamine on the blood pressure responses to intravenous noradrenaline in the conscious cat. Ordinate: the logarithm of the rise in blood pressure divided by the difference between the observed rise and the calculated maximum rise. Abscissa: the doses of intravenous noradrenaline in μg/kg plotted on a logarithmic scale. *Upper two lines:* ●——● noradrenaline alone, △——△ noradrenaline after 4 μg/kg thymoxamine. Each point is the mean of six observations on five cats. *Lower two lines*: ●——● noradrenaline alone, △——△ noradrenaline after 0·2 μg/kg phentolamine. Each point is the mean of seven observations on five cats.

and the blood pressure at a point when it had again stabilized after the injection (usually 5–8 min post-injection). The means of these differences, plotted as cumulative fall in blood pressure in mm Hg, against log cumulative dose of antagonist are shown with regression lines in Fig. 4. For both thymoxamine and phentolamine there was a linear relation between fall in blood pressure and the logarithm of the cumulative dose of antagonist. At the doses used, thymoxamine had a greater hypotensive effect than phentolamine. The regression lines did not differ significantly in slope ($P > 0{\cdot}9$).

Discussion

When the noradrenaline-induced blood pressure rises were plotted against log dose of noradrenaline (log x) the responses in the presence of the antagonists (y′) were moved to the right of the responses to noradrenaline alone (y) (Fig. 1). The first dose of the antagonists decreased the slope of the log dose-response line; in an isolated organ experiment this would suggest a non-competitive mode of antagonism. Increase in antagonist dose seemed to maintain the new slope, but the three log dose-response lines in the presence of antagonist were close together making further conclusions about the nature of the antagonism, from analogy with isolated organ experiments, more difficult. Thus it appears

that although a conventional log x versus y or y′ plot is known to be useful for analysing drug antagonisms on isolated tissues it yields limited information in the intact animal, in which the situation is more complex. Not only are injected drugs subjected to the processes of absorption, distribution and metabolism, but the measurements of arterial blood pressure are the resultants of the cardiac output and peripheral resistance influenced by the vasomotor compensatory reflexes in which noradrenaline released from sympathetic nerves plays a part. In the experiments here reported the situation was further complicated by the fact that the baseline blood pressure was itself lowered (further to activate the compensatory reflexes) by each succeeding dose of α-blocking agent, by an amount which was proportional to the logarithm of the cumulative dose of antagonist (Fig. 4). It is a common experience that a change in baseline of a measured function alters the extent to which that function may be modified

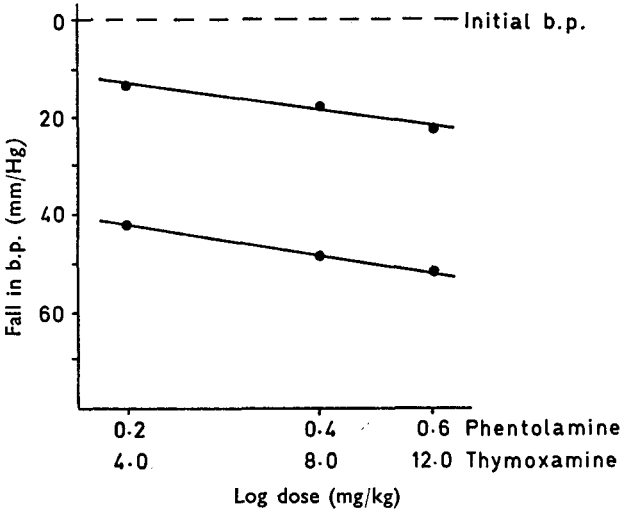

FIG. 4. The effect of thymoxamine or phentolamine on the blood pressure of the conscious cat. Ordinate: decrease in blood pressure in mm Hg (the resting blood pressure of the cat before antagonist minus the stable blood pressure after each succeeding dose of antagonist). Abscissa: the cumulative dose of the intravenously-administered α-blocking agent in mg/kg on a logarithmic scale. For phentolamine (upper line) each point is a mean of seven observations on five cats; for thymoxamine (lower line) each point is a mean of six observations on another five cats.

by drugs (Wilder, 1957a,b). The implication in this situation is that at a lower baseline blood pressure, the blood-pressure-raising effect of a dose of noradrenaline will be greater than the effect of the same dose of noradrenaline at a higher baseline blood pressure. Thus the closeness of the log dose-response lines in the presence of the antagonists could be, at least in part, a consequence of the effect of the antagonists on the baseline blood pressure.

Despite the complexity of the situation, Chen & Russell (1950) showed that by appropriate graphical treatment of the results it was possible to

analyse drug antagonism of the blood pressure responses to adrenaline in anaesthetized dogs. They used the graphical solutions of Lineweaver & Burk (1934) derived from the kinetics of enzyme-substrate and enzyme inhibitor combinations. For enzyme reactions, a plot of the reciprocal of the velocity of the reaction against the reciprocal of the substrate concentration yields a straight line. In the presence of competitive inhibitors the slope of the line is increased without change of intercept on the velocity co-ordinate, whereas non-competitive inhibitors increase the slope and raise the intercept. Chen & Russell (1950) showed in the anaesthetized dog that the slope of the lines obtained by plotting 1/dose against 1/response for adrenaline, increased in slope with cumulative increase in dose of yohimbine or with low doses of SY-28*, but the lines all had a common intercept with the line for adrenaline alone (by analogy with *in vitro* enzyme experiments this might indicate competitive antagonism) whereas the line for a large dose of SY-28 had a raised intercept on the 1/response co-ordinate (analogous with non-competitive antagonism in the enzyme experiments of Lineweaver & Burk, 1934).

When the present results were graphed as Lineweaver and Burk plots, 1/x versus 1/y or 1/y′ (Fig. 2), it was apparent that the first dose of thymoxamine or phentolamine increased the slope of the line and raised the intercept on the 1/response co-ordinate, indicating non-competitive antagonism. Thereafter, cumulative increases in antagonist dose gave lines showing progressively increased slopes but the intercepts were common with that of the line for the first dose of antagonist, indicating that further antagonism could be competitive. One exception to this general finding was seen when the total dose of phentolamine had reached 0·6 mg/kg. The response to the lowest dose of noradrenaline (0·1 μg/kg) was not linear with the responses to the other three doses. Such a breakdown of the linear relation between 1/x and 1/y′ was also found by Chen & Russell (1950) and by Matsumoto & Kumoi (1958) for acetylcholine-atropine combinations.

Matsumoto & Kumoi (1958) made a further analysis of the situation in which there was a change of intercept on the y axis and claimed that non-competitive antagonism could be differentiated from uncompetitive antagonism whereas such differentiation was not possible with log x versus y and y′ and 1/x versus 1/y and 1/y′ plots. By using the more complex co-ordinates log x and log $[y/(y_{max} - y)]$ or log $[y'/(y'_{max} - y')]$, derived from the considerations of Clark (1937) & Gaddum (1943),they showed that the position of the line in the presence of antagonist relative to that for the agonist alone allowed a further separation of the types of antagonism. When the present results for noradrenaline alone and for noradrenaline in the presence of the first dose of thymoxamine or phentolamine were treated in this way, the line for noradrenaline in the presence of the antagonist was moved to a position above and parallel to the line for noradrenaline alone (Fig. 3) conforming to the situation which Matsumoto & Kumoi (1958) held to be a combination of non-competitive and uncompetitive antagonism.

* *N*-(2-Bromoethyl)-*N*-ethylnaphth-1-ylmethylamine.

The broad similarities in the results of the quantitative analysis suggest that the mechanism of the noradrenaline antagonism exhibited by thymoxamine and phentolamine is the same. Thus it seems that a first dose of thymoxamine or phentolamine has a complex effect on the dynamics of a noradrenaline-induced blood pressure rise. This may in part be a reflection of the fact that for either antagonist the first dose had the greatest single effect in lowering the blood pressure (presumably by blockade of the effects of noradrenaline released from vasoconstrictor nerves) thereafter the dose increments effected smaller decreases in basal blood pressure. The differences between anaesthetized and unanaesthetized cats in their responses to catecholamines have been described by Thuránszky (1966a) and may be sufficient in themselves to account for the differences between our results with α-blocking agents and the results reported by Chen & Russell (1950) and those by Matsumoto & Kumoi (1958). It would appear from our experiments that after antagonism with an adrenergic blocking agent has been initiated in the conscious cat, or when the animal is anaesthetized, the antagonism has the characteristics associated with competitive blockade. These results do not provide direct evidence for the precise site of agonist-antagonist interaction. Although it is likely that most of the antagonism measured in these experiments was occurring at vascular α-receptors, the complexity of the factors controlling blood pressure in the conscious cat make it unlikely that α-blockade was the only mechanism involved. The antagonist may have been acting at sites other than the vascular α-receptors where the antagonism may be competitive or non-competitive. The basic assumption that the observed response was proportional to the number of receptors occupied by noradrenaline may not be valid. It is also possible that the different vascular beds in the animal have different affinities for the antagonists. Finally, as Lineweaver & Burk (1934) emphasized, mathematical manipulation of experimental data only indicates what mechanisms *may* be but not necessarily *are* involved in the situation examined.

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