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Mechanism of the antagonism of the hypotensive action of guanethidine by propranolol

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Summary

1. Propranolol antagonizes the hypotensive effect of guanethidine in renal hypertensive but not in adrenal demedullated renal hypertensive rats.

2. Guanethidine does not interfere with the release of catecholamines from the adrenal gland which follows splanchnic nerve stimulation in cats anaesthetized with pentobarbitone.

3. Guanethidine does not release or interfere with the release of catecholamines after the intravenous injection of physostigmine in the rat.

4. Propranolol antagonizes the antihypertensive effect of guanethidine by potentiating the vasopressor effects of catecholamines released from the adrenal glands during the induction stage of ether anaesthesia.

Introduction

The hypotensive activity of reserpine, guanethidine, hydralazine and α-methyldopa is blocked by propranolol in renal hypertensive rats (Brunner, Hedwall & Meier, 1965 ; Bein & Brunner, 1966). Prichard & Gillam (1969) have reported that propranolol reduces blood pressure in hypertensive patients and its effect is enhanced by guanethidine. It was therefore of interest to study the mechanism by which propranolol antagonizes the effects of other antihypertensive drugs and we have studied the interaction of guanethidine and propranolol in rats.

Methods

Renal hypertensive rats were prepared by the method of Goldblatt, Lynch, Hanzal & Summerville (1934). Rats having a stable systolic blood pressure of about 180 mmHg were used. Blood pressure was measured in mmHg (1 mmHg$\equiv$1·333 mbar) under light ether anaesthesia by tail plethysmography, 24, 48 and 72 h after treatment. The adrenal gland was enucleated by making a small incision and squeezing out the contents of the gland. In order to see whether all the medulla had been removed from the animals, histological examination as well as catecholamine estimation of the adrenal gland was carried out in some of the rats 5–6 weeks after adrenal enucleation. After adrenal enucleation, the rats were made hypertensive as described above. The rats were used for the experiments 5–6 weeks later when they had developed stable hypertension. The effect of guanethidine was then studied in both normal and enucleated renal hypertensive rats in the presence or

absence of propranolol. Guanethidine was given daily at a dosage of 20 and 30 mg/kg orally. Propranolol (0·25 and 1 mg/kg subcutaneously) was given daily 1 h before guanethidine administration.

Catecholamine release experiments were carried out in rats anaesthetized with urethane (1·5 g/kg). The main adrenal vein was cannulated and the total catecholamine released was measured in the venous effluent as noradrenaline during a control period, 0·5 h and 1 h after physostigmine 50 μg/kg. In these experiments both the jugular veins were cannulated, one for the injection of the drug and the other for continuous infusion of heparinized saline (0·1 ml/min). Adrenal venous blood was collected (approximately 0·5–0·7 ml in 30 min) in a chilled centrifuge tube. The plasma was separated (2,000 rev min^{-1}) and assayed for noradrenaline on the blood pressure of a pithed rat. As the volume of plasma was very small, the unknown plasma samples were bracketed between two and three doses of standard noradrenaline. In some experiments where the volume of plasma was more, two lines of a latin square were carried out for the assay. Guanethidine 20 mg/kg was given orally twice at 12 hourly intervals.

Drugs used. (−)-Noradrenaline-hydrogen tartrate, physostigmine salicylate, guanethidine sulphate and propranolol. The concentrations and doses of these drugs are expressed as their salts except for noradrenaline, which is expressed as the free base.

Results

The results of our experiments are shown in Tables 1 and 2. Guanethidine produced a significant fall of blood pressure in both normal and adrenal demedullated renal hypertensive rats (series 1, 2, 5 and 6). There was no significant difference between the fall in blood pressure produced by guanethidine in normal and that in demedullated renal hypertensive rats [series 1 and 2 (Table 1) and series 5 and 6 (Table 2)]. Pretreatment of the renal hypertensive rats with propranolol (0·25 mg/kg subcutaneously) completely blocked the hypotensive response to guanethidine. In the demedullated rats with renal hypertension (series 4, 8 and 9) propranolol did not antagonize the hypotensive effect of guanethidine. Significant falls in blood pressure occurred 48 and 72 h after treatment in series 8 and 9.

TABLE 1. *Effect of guanethidine (20 mg/kg orally daily) on the blood pressure of renal hypertensive rats (normal and demedullated)*

Series of renal hypertensive rats	Blood pressure (mmHg)			
	Before treatment	24 h after treatment	48 h after treatment	72 h after treatment
1. With medulla	190·60±6·47 (13)	155·10±8·63 (13)**	161·90±6·02 (13)**	160·00±7·85 (6)**
2. Demedullated	200·00±7·07 (14)	170·71±8·06 (14)**	171·00±6·48 (14)**	172·86±8·63 (7)*
3. With medulla+ propranolol (0·25 mg/kg) s.c. daily 1 h before guanethidine	192·69±7·84 (13)	190·15±5·60 (13)	201·69±6·12 (13)	190·00±5·48 (12)
4. Demedullated+ propranolol (0·25 mg/kg) s.c. daily 1 h before guanethidine	192·90±5·59 (14)	171·79±7·55 (14)*	169·71±6·91 (14)*	180·71±7·09 (7)

All values are the means±S.E.M. The figures in parentheses indicate the number of observations.
* $0·05>P>0·01$.
** $0·01>P>0·001$.

When a higher dose of guanethidine (30 mg/kg) was used, however, the fall in blood pressure was about 10% less in the demedullated renal rats than in normal renal rats.

In order to check whether guanethidine blocks the release of catecholamines from the adrenals following different stimuli, two types of experiments using two different types of adrenal stimulation were carried out. In one set, guanethidine (3 mg/kg intravenously) was used in the cat and it blocked the contraction of the nictitating membrane elicited by preganglionic stimulation. However the vasopressor effect of splanchnic nerve stimulation was unchanged (Fig. 1).

TABLE 2. *Effect of guanethidine (30 mg/kg orally daily) on the blood pressure response of renal hypertensive rats normal and demedullated*

Series of renal hypertensive rats	Blood pressure (mmHg) Before treatment	24 h after treatment	48 h after treatment	72 h after treatment
5. With medulla	204·58±7·89 (12)	157·91±7·00 (12)***	133·33±8·75 (12)***	131·66±8·78 (12)***
6. Demedullated	212·00±9·60 (5)	181·00±11·55 (5)*	146·00±11·33 (5)***	152·00±11·91 (5)***
7.† With medulla +propranolol	211·00±10·68 (6)	201·67±13·39 (6)	198·30±11·97 (6)	190·80±6·88 (6)
8.† Demedullated +propranolol	210·00±15·25 (5)	176·00±13·17 (5)	159·00±5·79 (5)*	162·00±6·44 (5)*
9.† Demedullated +propranolol	203·30±8·72 (8)	187·50±7·04 (6)	166·67±5·11 (6)**	165·00±10·80 (6)*

All values are the means±S.E.M. The figures in parentheses indicate the number of observations.
* $0{\cdot}05 > P > 0{\cdot}01$.
** $0{\cdot}01 > P > 0{\cdot}001$.
*** $0{\cdot}001 > P$.
† In series 7 and 8 propranolol 0·25 mg/kg subcutaneously and in series 9, 1 mg/kg subcutaneously, was given daily 1 h before guanethidine.

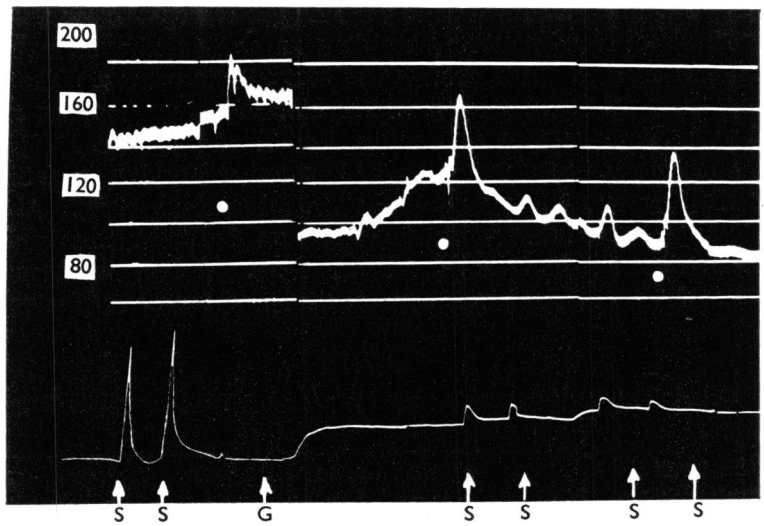

FIG. 1. Blood pressure (upper record) and nictitating membrane (lower record) of an anaesthetized cat (pentobarbitone anaesthesia, 45 mg/kg intraperitoneally). At white dots, splanchnic nerve stimulations (4 V, 32 Hz, 0·46 ms); at S, preganglionic stimulation of the cervical sympathetic chain (3·2 V, 32 Hz, 0·46 ms). At G, guanethidine 3 mg/kg was given intravenously. Note that the effect on the nictitating membrane is blocked completely whereas the effect of splanchnic nerve stimulation is not blocked by guanethidine. (Stimulation repeated 1 and 2 h after guanethidine.)

In another set of experiments an attempt was made to measure quantitatively the release of catecholamines in the rat in response to the intravenous injection of physostigmine (50 μg/kg). Physostigmine causes a marked increase in the catecholamine output of the rat adrenal gland (Kaul & Grewal, 1968). In the present series of experiments we have confirmed these results and shown that guanethidine pretreatment does not reduce the physostigmine induced increase in catecholamine output from the adrenal gland (Table 3).

Discussion

Our results show that propranolol does not antagonize the antihypertensive effect of guanethidine in adrenal demedullated rats but it blocks the effect of guanethidine in renal hypertensive rats with the adrenal medulla intact (Tables 1 and 2). These findings suggest that release of catecholamines from the adrenal glands may be involved in annulling the antihypertensive effect of guanethidine after blockade of β-adrenoceptors.

Guanethidine is known to produce its antihypertensive effect by blocking adrenergic transmission. This is associated with a depletion of neurotransmitter from adrenergic nerve endings resulting in prolonged sympathetic atony (Maxwell, Mull & Plummer, 1959; Maxwell, Plummer, Povalski & Schneider, 1960; Page & Dustan, 1959; Cass, Kuntzman & Brodie, 1960). However, it does not reduce the catecholamine content (Cass *et al.*, 1960) nor discharge catecholamines from adrenal glands (Butterfield & Richardson, 1961; Athos, McHugh, Fineberg & Hilton, 1962). Therefore, the difference between the adrenal demedullated and the normal rats was not due to the release of catecholamines by guanethidine.

It seems that release of catecholamines from the adrenal gland in the presence of β-adrenoceptor blocking drugs is great enough to block the hypotensive effect of guanethidine. The question arises as to the stimulus responsible for this release. The measurements of blood pressure were done under light ether anaesthesia and during the induction stage there was marked sympathetic activity. The released adrenaline in the presence of β-adrenoceptor blocking drugs becomes markedly pressor as its vasodilator component is antagonized (Grewal & Kaul, 1969). Potentiation of the effects of catecholamines by β-adrenoceptor blocking drugs have been reported in the literature (Nickerson, 1965; Grewal & Kaul, 1969). Abboud & Eckstein (1962) postulated that guanethidine acts on β-adrenoceptors and this to some extent contributes to its vasodilator effect. Propranolol might antagonize this action of guanethidine. Therefore it appears that β-adrenoceptor blocking drugs

TABLE 3. *Catecholamine output from the adrenal vein in rats given physostigmine* (50 μg/kg)

		0 h		0·5 h		1 h	
Series	Treatment	ng/total	ng/min	ng/total	ng/min	ng/total	ng/min
A.	Physostigmine	65·43 ±8·81 (37)	3·10 ±0·45 (33)	133·63 ±22·3 (20)	4·93 ±0·98 (17)	171·20 ±20·8 (27)	6·65 ±1·08 (22)
B.	Guanethidine* + Physostigmine	60·9 ±12·54 (11)	2·05 ±0·56 (11)	—	—	157·0 ±31·08 (10)	4·96 ±1·1 (10)

All values are the mean±S.E.M. Figures in parentheses indicate the number of observations.
* Guanethidine was given 20 mg/kg orally twice with 12 h interval.

antagonize the antihypertensive effect of guanethidine mainly by potentiating the vasopressor effects of catecholamines released from the adrenal glands and possibly to a small extent also by blocking the β-adrenoceptor stimulation by guanethidine.

It was not possible to show this antagonism in the presence of other anaesthetics such as pentobarbitone which reduce the blood pressure of hypertensive rats to almost normotensive levels. The antagonism of guanethidine by propranolol appears to occur only in certain experimental conditions when marked release of catecholamine from the adrenal gland takes place. The mechanism by which propranolol enhances the antihypertensive effects of guanethidine (Prichard & Gillam, 1969) is therefore unrelated.

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