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MODIFICATION OF PRESSOR EFFECTS OF SOME VASOACTIVE POLYPEPTIDES IN THE RAT BY GUANETHIDINE, PROPRANOLOL AND RELATED AGENTS

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There have been numerous reports in the literature of pressor responses being obtained, in the rat as well as in other animal species, after intravenous administration of polypeptides normally possessing marked vasodilator properties. Thus bradykinin and lys. bradykinin (kallidin) give a biphasic response (hypotensive-hypertensive), or a purely hypertensive one, in nephrectomized rats (Croxatto & Belmar, 1962), in rats with low initial blood pressure levels (Parratt, 1964) and rats rendered hypotensive by ganglion blocking substances (Croxatto & Belmar, 1962; Rosas, Montague, Gross & Bohr, 1965), by acute haemorrhage, by overdose of pentobarbitone sodium and by injection of large doses of the vasodilator polypeptides (Parratt, 1964). Bradykinin also gives a biphasic response in guanethidine-pretreated rats (Miele & De Natale, 1966) and in pregnant cats (Parratt, 1964) or cats treated with desmethylimipramine (Miele, 1966b).

Eledoisin and physalaemin produce hypertensive responses in pithed rats, in rats with low blood pressure levels induced by ganglion blocking drugs, and in intact or decapitated chickens (Erspamer & Glaesser, 1963; Stürmer & Berde, 1963; Nakano, 1964; Parratt, 1964; Bertaccini, Cei & Erspamer, 1965). Since the hypertensive effect becomes abolished or considerably reduced by bilateral adrenalectomy, by pretreatment with reserpine and by acute administration of α-receptor blocking substances, it might well be the result of the liberation of catecholamines from the adrenal medulla and/or from adrenergic nerve endings.

Direct proof of the liberation of catecholamines from the adrenal medulla by vasoactive polypeptides has been given for bradykinin by Lecomte, Troquet & Dresse (1961); Feldberg & Lewis (1964, 1965); Staszewska-Barczak & Vane (1964) and Vogt (1965) and for lys. bradykinin by Feldberg & Lewis (1965) and Staszewska-Barczak & Vane (1964). Eledoisin has been found to be scarcely active (Staszewska-Barczak & Vane, 1964) or completely inactive (Lewis & Reit, 1966). We have found no data on this aspect of physalaemin in the literature.

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We have investigated in the rat the effects of guanethidine on the blood pressure response to these vasoactive polypeptides, since guanethidine, as opposed to reserpine, provokes catecholamine depletion only at the adrenergic neurone level, leaving unaffected those of the adrenal medulla (Cass, Kuntzman & Brodie, 1960; Cession-Fossion, 1965) and potentiates the cardiovascular effects of exogenous catecholamines and those liberated from the adrenal medulla (Green, 1962; Miele, 1966a).

METHODS

The tests have been conducted on male rats of the Wistar-Morini strain, with body weight ranging from 380 to 440 g.

The animals were anaesthetized with urethane (1 g/kg intraperitoneally), then immobilized on thermoregulated operating tables. They were given artificial respiration (Miniature Pump Palmer) at constant rate and volume and carotid blood pressure was recorded by means of a polythene cannula connected to a mercury manometer (Palmer Condon type).

Pithed rats were prepared under light ether anaesthesia. After the destruction of the spinal cord between C4 and C6, the animals were immediately artificially ventilated, and blood pressure was recorded as described above.

Bilateral adrenalectomy was performed by the dorso-lumbar approach, according to Farris & Griffith (1949).

For intravenous injection, a polythene cannula was tied in the jugular vein.

The following drugs were used: synthetic bradykinin (BRS 640-Sandoz); synthetic lys. bradykinin (kallidin) (KL 698-Sandoz); synthetic eledoisin (F.I. 6225-TF/OCOA Farmitalia); synthetic physalaemin (F.I. 6422 TF/CCRD Farmitalia); adrenaline HCl (Rivetti); guanethidine sulphate (reagent grade, Gianni); pronethalol (or nethalide) HCl (Alderlin I.C.I.); propranolol HCl (Inderal, I.C.I.); dichloroisoproterenol (or DCI) HCl (Simes); d-amphetamine sulphate (ISI-Napoli); reserpine (Serpasil, Ciba); phentolamine HCl (Regitin, Ciba); azamethonium dichloride (Pendiomid, Ciba).

All of the drugs were diluted or dissolved in 0.9% saline solution.

Bradykinin, lys. bradykinin, eledoisin and physalaemin were always injected, unless specified otherwise, in a volume of 0.2 ml./animal, followed by 0.2 ml. of saline solution.

The other drugs were administered in a volume of liquid /kg or /animal as specified below, and were followed each time by 0.2 ml. of saline solution. The doses are expressed in terms of their respective salts.

Pretreatment with guanethidine. The following two procedures were used for pretreatment with guanethidine. The first consisted of two injections of guanethidine 10 mg/kg given intraperitoneally, 24 and 6 hr before beginning the recording of arterial pressure. The second procedure consisted of intraperitoneal injections of guanethidine 5 mg/kg/day for five consecutive days. The last injection was given 8 hr before beginning the acute experiment.

The above two procedures are more than sufficient to effect a depletion of catecholamines from the peripheral sympathetic nerve endings without affecting those from the adrenal medulla (Cass *et al.*, 1960; Cession-Fossion, 1965; Chang, Costa & Brodie, 1965).

RESULTS

Effects of acute intravenous administration of guanethidine

The intravenous administration of guanethidine in doses of 5 or 10 mg/kg in 1 ml. of liquid/kg was followed by a considerable fall in the blood pressure. The initial blood pressure varied from 60 to 105 mm Hg. The degree of the fall was roughly proportional to the initial level.

Bradykinin. During hypotension from guanethidine, the hypotensive response to bradykinin (5 γ/kg) was usually reduced or abolished, in proportion to the fall of the blood pressure. Usually, after 5–30 min for a dose of 10 mg/kg guanethidine and 3–15 min for a dose of 5 mg/kg, the response to bradykinin assumed the typical biphasic aspect, with a marked prevalence of the hypertensive phase.

The pressor response to adrenaline (doses of 0.25–0.6–2.5 γ/kg in 0.2 ml./animal) appeared to be potentiated immediately by the administration of guanethidine.

The administration of a β-receptor blocking substance, such as propranolol, pronethalol or DCI, either before or at the same time as guanethidine (in doses varying from 1.25 to 2.5 mg/kg in 0.5 ml/kg), caused abolition of the lag-phase and the response to bradykinin quickly assumed the typical biphasic aspect. There was a potentiation of the hypertensive phase in the response to bradykinin if the β-receptor blocker was administered after guanethidine (Fig. 1). In such an experiment propranolol was more active than pronethalol while DCI was scarcely active.

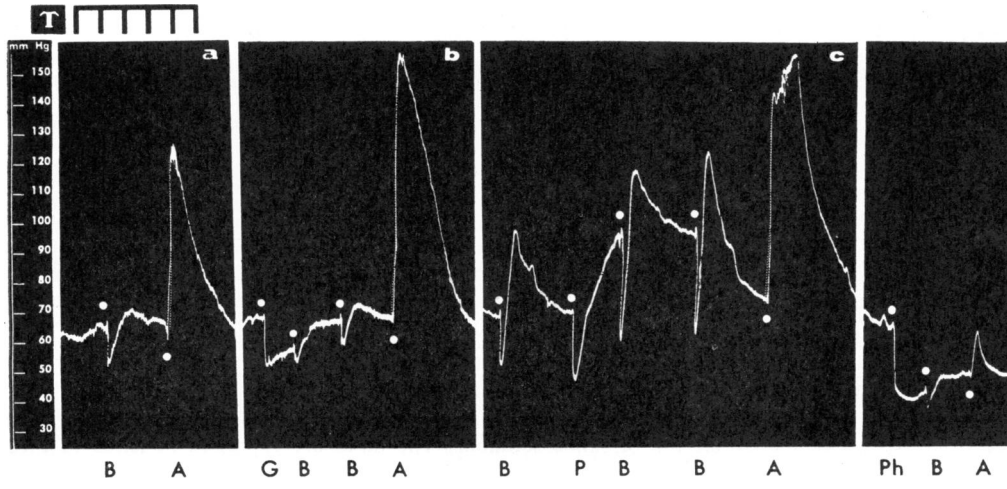

Fig. 1. Effects of intravenous administration of guanethidine and propranolol on the blood pressure responses to bradykinin and to adrenaline. B=bradykinin 5 γ/kg; A=adrenaline 2.5 γ/kg; G=guanethidine 5 mg/kg; P=propranolol 2.5 mg/kg; Ph=phentolamine 2.5 mg/kg; T=time in min. (a) and (b)=pressor responses to bradykinin and to adrenaline before and after guanethidine; (c)=first response to bradykinin evoked 12 min after guanethidine; (d)=response to bradykinin and to adrenaline after phentolamine.

The pressor response to adrenaline also became enhanced after administration of propranolol or pronethalol alone (but scarcely after DCI). The response to bradykinin was modified by propranolol and the duration of the hypotension was shortened. In some experiments a weak secondary hypertensive phase appeared.

The intraperitoneal administration of d-amphetamine in doses of 2–2.5 mg/kg in 2 ml./kg, 20–45 min before guanethidine, was neither able to antagonize the lag-phase nor enhance the hypertensive phase of the response to bradykinin.

Eledoisin and physalaemin. In rats with high blood pressure the intravenous administration of guanethidine, either alone or with β-receptor blocker at the doses

specified above usually enhanced the hypotensive responses to eledoisin (0.5–1–2.5 γ/kg) and to physalaemin (0.1–1–2.5–5 γ/kg). However, in spite of the marked fall in blood pressure produced by intravenous guanethidine, it was never possible to observe a secondary hypertensive response to eledoisin and physalaemin (Fig. 2).

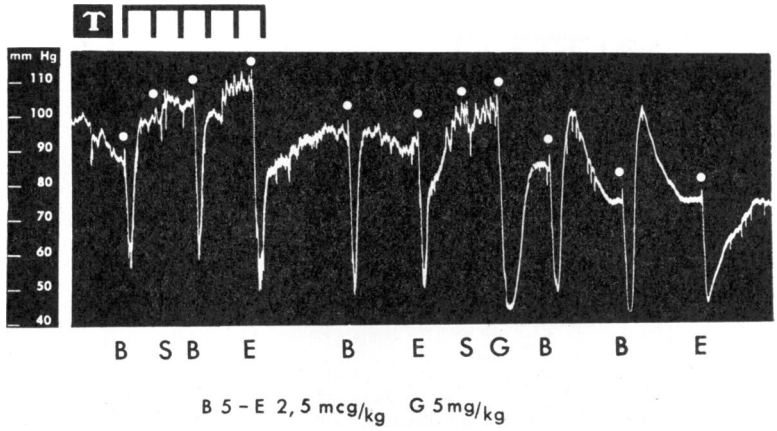

Fig. 2. Effects of intravenous administration of guanethidine on the blood pressure responses to bradykinin and to eledoisin. B=bradykinin 5 γ/kg; S=saline solution 0.2 ml/animal; E=eledoisin 2.5 γ/kg; G=guanethidine 5 mg/kg; T=time in min.

In animals with initially low blood pressure in which the response to eledoisin and physalaemin was predominantly hypertensive, intravenous administration of guanethidine, abolished the hypertensive phase and enhanced the hypotensive one (Fig. 3).

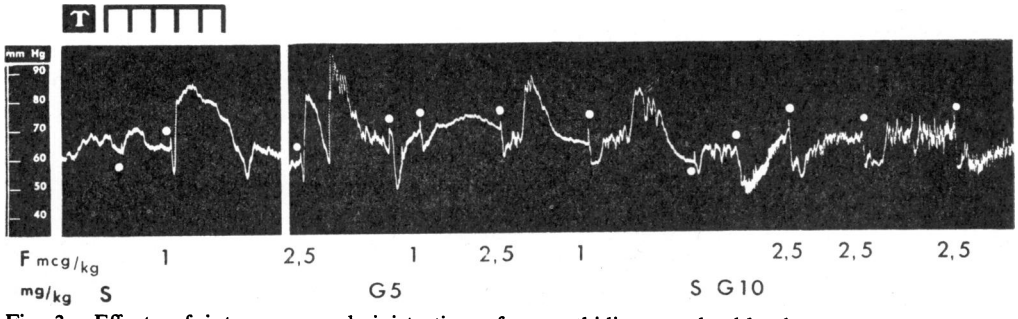

Fig. 3. Effects of intravenous administration of guanethidine on the blood pressure responses to physalaemin. S=saline solution 0.2 ml./animal; F=physalaemin in γ/kg; G=guanethidine in mg/kg.

Effects of pretreatment with guanethidine

The blood pressure of these animals was almost the same as that in rats which were not pretreated (60–100 mm Hg).

Bradykinin and lys. bradykinin. The pressor response to bradykinin and to lys. bradykinin (5 γ/kg) was typically biphasic. The hypotensive phase was sometimes more marked than that observed in the untreated controls but the hypertensive phase usually predominated.

The secondary hypertensive phase of the pressor response to bradykinin and to lys. bradykinin was abolished or strongly reduced by the administration of an α-receptor blocker (phentolamine 1–2.5 mg/kg intravenously in 0.2 ml./animal), or by bilateral adrenalectomy during the test. Furthermore, this secondary hypertensive phase was completely lacking in previously adrenalectomized rats (Fig. 4).

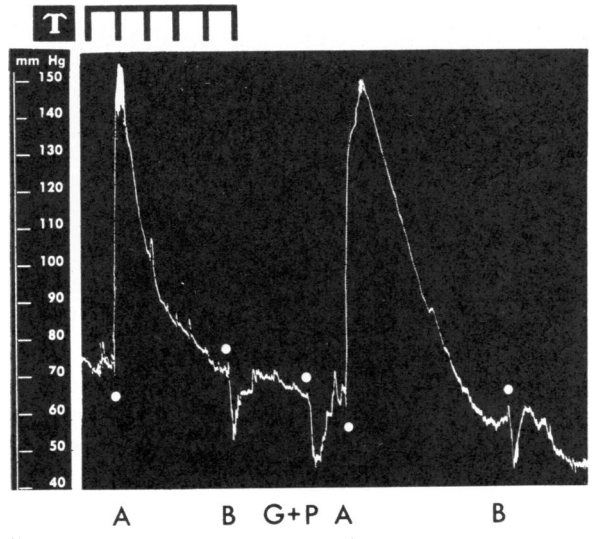

Fig. 4. Effects of bilateral adrenalectomy on the blood pressure responses to bradykinin and adrenaline in a rat pretreated with guanethidine (first procedure). The bilateral adrenalectomy was performed 30 min before the start of the registration of the blood pressure. A=adrenaline 0.6 γ/kg; B=bradykinin 5 γ/kg; G+P=simultaneous intravenous administration of guanethidine 5 and propranolol 2.5 mg/kg; T=time in min.

On the other hand, the administration of a β-receptor blocker (pronethalol or propranolol) either alone or with guanethidine, resulted in a marked enhancement of the hypertensive phase.

Eledoisin and physalaemin. The responses to eledoisin and physalaemin (doses ranging from 0.1 to 5 γ/kg) were purely hypotensive.

These responses appeared to be greater than those in normotensive controls. In no case was a secondary hypertensive phase observed in the blood pressure response to these two polypeptides.

Azamethonium. In a few of the guanethidine pretreated animals where the initial blood pressure was almost the same as in untreated rats, the administration of a ganglion blocking substance (azamethonium dichloride, 2.5–5 mg/kg intravenously in 1 ml./kg) was followed by a conspicuous fall in the blood pressure. During this hypotension, bradykinin preserved the typical biphasic pattern. Physalaemin always gave a more prolonged hypotensive response. Eledoisin also usually gave a purely hypotensive response.

In some experiments it was possible to show a hypertensive response to eledoisin which was smaller than that obtained with bradykinin (Fig. 5).

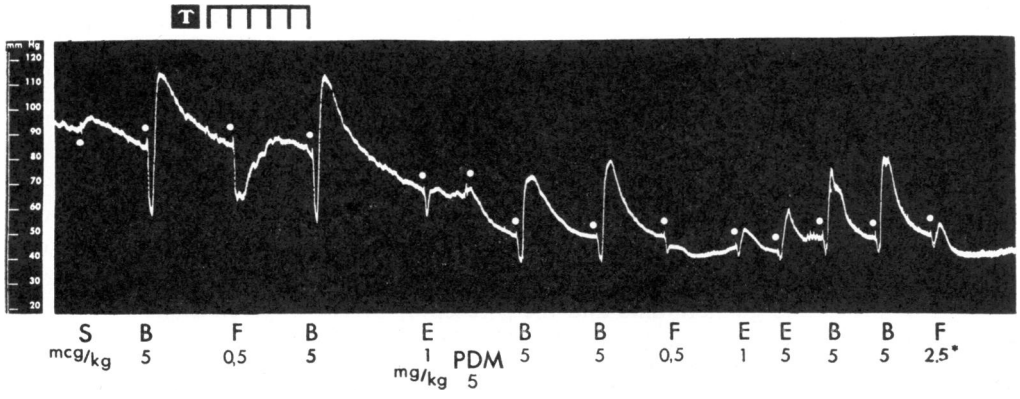

Fig. 5. Effects of the intravenous administration of azamethonium on the blood pressure responses to bradykinin, to eledoisin and to physalaemin in a rat pretreated with guanethidine (first procedure). S=saline solution 0.2 ml./animal; B=bradykinin; E=eledoisin and F=physalaemin in γ/kg. The last injection of physalaemin (2.5*) was made in 1 ml. of liquid/animal in a time of 30 sec. PDM=azamethonium dichloride 5 mg/kg. T=time in min.

Pithing. In pithed animals pretreated with guanethidine, the blood pressure levels varied from 60 to 70 mm Hg. The responses to bradykinin and lys. bradykinin were usually biphasic, whereas those to eledoisin and physalaemin were always hypotensive. Even in these animals, the simultaneous administration of propranolol and guanethidine (doses of 1.25 and 2.5 mg/kg respectively in 0.3 ml./animal) resulted in enhancement of the secondary hypertensive phase of the response to bradykinin and lys. bradykinin, while the responses to eledoisin and physalaemin were not changed.

DISCUSSION

The acute intravenous administration of guanethidine to the normal rat produces hypotension, modifies the vascular response to bradykinin and enhances the hypotensive blood pressure responses to eledoisin and physalaemin.

In addition, in rats with spontaneous hypotension, guanethidine abolishes the hypertensive phase of the response to eledoisin and physalaemin. It is known that guanethidine hinders the liberation of the neurotransmitter from adrenergic nerves both in response to stimulation of the nerve fibres and after the administration of sympathomimetic amines which act by a tyramine-like mechanism (Maxwell, Plummer, Povalski & Schneider, 1960; Abercrombie & Davies, 1963).

Our results confirm that bradykinin and lys. bradykinin liberate catecholamines from the adrenal medulla, whereas eledoisin and physalaemin probably liberate catecholamines from adrenergic neurones perhaps by a tyramine-like mechanism. This hypothesis is supported by experiments conducted in guanethidine-pretreated rats, in which guanethidine had selectively depleted catecholamines from adrenergic neurones, but not from the adrenal medulla (Cass *et al.*, 1960; Cession-Fossion, 1965; Chang *et al.*, 1965). In these animals the response to bradykinin and lys. bradykinin is usually biphasic, whereas the response to eledoisin and to physalaemin is usually hypotensive, even if the blood pressure has been lowered by the administration of azamethonium.

However, eledoisin under certain conditions such as after a ganglionic blocking agent, can cause a slight hypertensive effect, probably as the result of release of small quantities of catecholamines from the adrenal medulla.

The secondary hypertensive response to bradykinin and lys. bradykinin in the guanethidine pretreated rat is without doubt due to adrenaline released from the adrenal glands (Feldberg & Lewis, 1964). This is indicated in the present experiments by the following observations: (a) the hypertensive phase is absent following the administration of an α-adrenergic blocker (phentolamine) or by acute or chronic bilateral adrenalectomy (Miele & De Natale, 1966); (b) an enhancement of this secondary hypertensive phase is effected by drugs like guanethidine and the β-receptor blockers (given alone or together) which enhance the pressor effects of catecholamines. As far as the β-receptor blockers are concerned Shanks (1966) has shown in dogs that enhancement occurs only of adrenaline. The lag-phase which occurs after the intravenous administration of guanethidine, before the appearance of the hypotensive phase of the response to bradykinin, is absent after the administration of β-receptor blockers (propranolol in particular). The lag-phase cannot be related to the rapid blockade of the adrenergic neurone by guanethidine, because d-amphetamine, a specific antagonist of such an effect produced by guanethidine (Day & Rand, 1963), is inactive in abolishing it.

SUMMARY

1. The depressor response to bradykinin and lys. bradykinin in rats is converted to a biphasic response after injection of guanethidine. The resulting pressor response is inhibited by administration of α-receptor blocker or by bilateral adrenalectomy and enhanced after injection of β-receptor blocker.

2. The hypertensive effect sometimes seen after eledoisin and physalaemin is abolished by guanethidine and the hypotension is usually enhanced.

3. It seems likely that in rats the hypertension produced by bradykinin and lys. bradykinin is mediated mainly *via* adrenaline released from the adrenal glands, while the hypertensive response produced by eledoisin and physalaemin is mediated by catecholamines originating primarily from the adrenergic neurones.

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