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EFFECTS OF GUANETHIDINE ON HISTAMINE RELEASE DURING REFLEX VASODILATATION IN THE DOG

M. CONDORELLI, G. RASETTI, F. RENGO, B. RICCIARDELLI, L. SACCÀ, B. TRIMARCO & M. VOLPE

Istituto di Patologia Speciale Medica e Metodologia Clinica, 2^ Facoltà di Medicina e Chirurgia dell 'Universita' di Napoli, Italy.

1 The effects of guanethidine pretreatment on the release of [^{14}C]-histamine during the reflex vasodilatation induced in the atropinized gracilis muscle by rapid intravenous administration of noradrenaline, were studied in dogs.

2 After guanethidine treatment the haemodynamic reflex response was completely abolished and no appreciable modification of [^{14}C]-histamine release from the gracilis muscle following intravenous noradrenaline was observed.

3 These results suggest the hypothesis that the withdrawal of the sympathetic discharge represents the mechanism of histamine release during the reflex vasodilatation. Therefore, guanethidine would suppress both the passive and the histaminergic component of the baroreceptor reflex through the abolition of the sympathetic tone.

Introduction

In previous work, designed to demonstrate the presence of afferents to vasodilator cholinergic encephalic centres in the sinus nerve of the dog, we observed that intravenous guanethidine administration reduced the vasodilatation in the perfused hind limb evoked by stimulation of the carotid sinus nerves and that intra-arterial atropine completely abolished the residual vasodilatation (Rengo, Perez, Chiariello, De Caprio, Saccà, Trimarco & Condorelli, 1976).

Based on previous observations suggesting that histamine participates as an active component in the genesis of reflex vasodilatation (Beck & Brody, 1961; Beck, 1965; Brody, 1966; 1968; Heitz, Shaffer & Brody, 1970), we interpreted the abolition of the reflex vasodilatation after treatment with guanethidine plus atropine as follows: while atropine blocks the cholinergic component alone, guanethidine not only abolishes the sympathetic vasoconstrictor tone but also blocks histamine release. Such an interpretation is also in agreement with the observation that reserpine, which shares with guanethidine a catecholamine depleting action, inhibits histamine release during reflex vasodilatation (Giotti, Guidotti, Mannaioni & Zilletti, 1966).

More recently, Weaver & Gebber (1974), recording electrical activity from the lumbar sympathetic nerve, immediately central to where section eliminated the active dilatation, observed only sympathetic inhibition in response to stimulation of the afferent vagus nerve or medullary depressor sites from which active dilatation had been produced. These authors, therefore, conclude that reflex vasodilatation does not result from excitation of any dilator pathway and cast doubt on the role of histamine in the genesis of the baroreceptor reflex. This interpretation would also be in agreement with a previous hypothesis of Glick, Wechsler & Epstein (1968), who suggested that antihistamines might reduce reflex vasodilatation by interfering with the reuptake of noradrenaline into adrenergic nerve terminals, rather than by antagonising the effects of histamine itself.

In the light of these data, the present work was mainly undertaken in order to establish whether guanethidine is really able to block the reflex vasodilatation by suppressing histamine release from the perfused atropinized gracilis muscle of the dog.

Material and Methods

Experiments were performed on mongrel dogs of either sex, weighing 10–20 kg. Anaesthesia was induced with sodium thiopentone (Farmotal, Farmitalia) (30 mg/kg, i.v.); additional doses of 30–50 mg were given as required. The trachea was intubated and artificial ventilation was performed at a

rate of 13–16 cycles per min after skeletal muscle relaxation induced by succinylcholine (Midarine, Wellcome) (0.2 mg/kg, i.v.). Body temperature was periodically measured and maintained between 37–37.5°C. The arterial P_{O_2} was maintained above 90 mmHg and P_{CO_2} below 35 mmHg; arterial pH was about 7.40. The arterial P_{O_2}, P_{CO_2} and pH were measured by a blood gas analyzer (Corning EEL 165).

The perfused region was the gracilis muscle isolated according to the technique of Renkin & Rosel (1962). The perfusion was performed at constant flow with blood of the same animal by means of a peristaltic pump (Sigmamotor T6S). For this purpose after administration of heparin (Liquemin, Roche) (5 mg/kg, i.v.), a polyethylene catheter was introduced through the external iliac artery into the abdominal aorta and blood was reinfused, by the perfusion pump, into the gracilis main artery. Under such conditions, changes in vascular tone of the perfused region were reflected by proportional changes in perfusion pressure. Blood flow was adjusted to give a perfusion pressure approximately equal to the systemic blood pressure and was left unchanged throughout the course of the experiment. Perfusion pressure was measured through a T connector interposed between the pump and the perfused region, while arterial blood pressure was recorded by a catheter placed in the thoracic aorta via the contralateral femoral artery. A Battaglia-Rangoni multichannel polygraph and pressure transducers were used.

Endogenous substances released by systemic hypertension and arterial catecholamines were delayed in their arrival to the perfused gracilis muscle because of the long time required to pass through the pump and therefore did not exert any influence on the recording of the haemodynamic phenomena.

The vein of the gracilis muscle was cannulated and the effluent blood was collected into a beaker and was not returned to the experimental animal so that drugs administered intra-arterially to the gracilis muscle had only peripheral effects since they never reached the systemic circulation. Heparin-treated blood of another dog was infused into the contralateral femoral vein at the same rate as the blood flowing from the gracilis vein.

At least 20 min were allowed after the completion of all surgical procedures before beginning the experiment.

In five dogs baroreceptor stimulation was carried out by rapid intravenous injection of noradrenaline (2 μg/kg). Since the participation of the cholinergic system in the genesis of the reflex vasodilatation has been recently demonstrated (Takeuchi & Manning, 1971; 1973; Ellison & Zanchetti, 1973; Rengo, Chiariello, De Caprio, Saccà, Trimarco, Perez & Condorelli, 1975; Chiariello, Condorelli, De Caprio, Rengo, Saccà & Trimarco, 1976), the muscle was atropinized in order to abolish this vasodilator component. After the intra-arterial administration of atropine (0.3 mg), the completeness of the cholinergic blockade was tested by the abolition of the haemodynamic response to the intra-arterial injection of acetylcholine (0.5 μg); then, the first reflex response was evoked by intravenous noradrenaline administration. In the same way, after intravenous administration of guanethidine, the effectiveness of the adrenergic blockade was first ascertained by the lack of change in systemic and perfusion pressure following the occlusion of the carotid arteries, and then a second response to the intravenous injection of noradrenaline was also evaluated. Responses were elicited at 20 min intervals.

To confirm the specificity of the pharmacological blockade of atropine and guanethidine, the haemodynamic response to intra-arterial administration of noradrenaline (0.1 μg) and sodium nitrite (0.15 mg), before and after administration of atropine and atropine plus guanethidine was evaluated in five experiments.

In order to investigate whether both reflex vasodilatation and the increase of histamine release induced by the intravenous injection of noradrenaline could be ascribed to baroreceptor stimulation, five experiments were performed in which the reflex was evoked before and after surgical selective baroreceptor denervation.

In the experiments in which the release of [^{14}C]-histamine was evaluated from the gracilis muscle during the baroreceptor reflex, 25 μCi of histamine [2 ring-^{14}C]-dihydrochloride (specific activity 59 mCi/mM, Amersham) in 1 ml of 0.9% w/v NaCl solution (saline) was infused into the perfusion tubing over a 5 min period. The radioactive venous effluent, collected only during the infusion of radiolabelled histamine, was reperfused into the gracilis muscle through a side arm on the inflow side of the Sigmamotor pump. A 40 min period was allowed for the radioactive material in the venous effluent to reach a stable level (Brody, 1966). All samples were collected in chilled tubes containing 6 ml of 0.6 N $HClO_4$.

Preresponse samples were collected in each case to determine the basal levels of radioactive materials in the blood. During the various responses studied, 30 s samples were collected continuously for a total of 2 minutes.

Total radioactivity was measured by mixing an aliquot of the perchloric acid extract with 10 ml of Instagel (Packard Instrument). [^{14}C]-histamine was extracted according to the method of Snyder, Axelrod & Bauer (1964). The method used in this study give a fairly accurate measurement of histamine output, as shown by Brody (1966).

To exclude the possibility that time-dependent modifications in the increase of [^{14}C]-histamine release

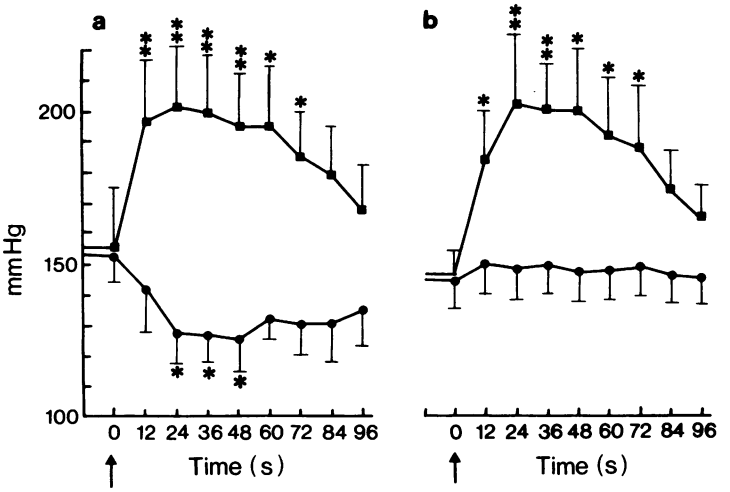

Figure 1 Effects of intravenous injection of noradrenaline before (a) and after (b) intravenous administration of guanethidine: (■) blood pressure; (●) perfusion pressure. Each point represents mean of 5 observations. Vertical lines show s.e. mean. Arrows indicate the time of noradrenaline injection. Statistical comparison is made between 0 time and each point using *t* test for paired samples.
$^{*}P < 0.05$; $^{**}P < 0.01$.

during the vasodilator response might occur, five experiments were performed in which two reflexes were evoked by intravenous injection of noradrenaline (2 μg/kg) at 30 min intervals without any pharmacological treatment.

The following drugs were used: atropine (Lancellotti); guanethidine (Ismelin, Ciba) (8 mg/kg, i.v.); acetylcholine (Koch-Light) (0.5 μg, i.a.); noradrenaline (Noradrec, Recordati).

Statistical analysis was conducted by standard techniques (Snedecor & Cochran, 1967).

Results

The intravenous noradrenaline injection induced a systemic hypertension and a statistically significant decrease of perfusion pressure in the atropinized gracilis muscle circulation (Figure 1a). After guanethidine treatment, the intravenous injection of noradrenaline did not induce any haemodynamic change in the perfused gracilis, while the systemic hypertensive response was unmodified (Figure 1b).

On the other hand, neither atropine nor atropine plus guanethidine modified the response to the intra-arterial administration of noradrenaline and sodium nitrite (Table 1).

The results of the experiments with labelled histamine are summarized in Figure 2. It is clear that during the reflex vasodilatation secondary to intravenous noradrenaline, both total radioactivity and [^{14}C]-histamine significantly increased in the blood flowing from the gracilis muscle. After guanethidine treatment, no appreciable modification of [^{14}C]-histamine release was observed. Moreover, the comparison between the release of [^{14}C]-histamine due to reflex vasodilatation at corresponding times before and after guanethidine administration, shows that there is a significant difference between the release rates (Figure 2).

On the other hand, in the experiments in which the reflex was evoked at 30 min intervals without any pharmacological treatment, no statistically significant change in the increase of [^{14}C]-histamine release during the reflex responses could be demonstrated by covariance analysis (Table 2).

The results of the experiments performed to establish the selectiveness of the baroreceptor stimulation indicate that the selective baroreceptor denervation is able to abolish both the dilatation and the increase in histamine release (Table 3).

Discussion

The observation that intravenous guanethidine administration completely abolishes the reflex vasodilatation confirms our previous results regarding the abolition of the reflex response to the electrical stimulation of the sinus nerves in atropinized hind limb (Rengo *et al.*, 1976).

On the other hand, the finding that neither atropine nor guanethidine induced any modification of the haemodynamic response to the intra-arterial injection

Table 1 Effects of intra-arterial noradrenaline and sodium nitrite on blood pressure and perfusion pressure in gracilis of dog

Treatment		*Seconds after drug injection* 0	36	72
Noradrenaline alone	BP	186 ± 13	186 ± 13	186 ± 13
	PP	199 ± 8	228 ± 9	213 ± 9
			$P < 0.005$	$P < 0.05$
After atropine	BP	186 ± 13	186 ± 13	186 ± 13
	PP	198 ± 8	229 ± 9	214 ± 9
			$P < 0.001$	$P < 0.025$
After atropine plus guanethidine	BP	186 ± 13	186 ± 13	186 ± 13
	PP	211 ± 8	235 ± 10	222 ± 10
			$P < 0.01$	$P < 0.05$
Sodium nitrite alone	BP	178 ± 3	178 ± 3	178 ± 3
	PP	189 ± 11	150 ± 10	180 ± 12
			$P < 0.01$	$P < 0.02$
After atropine	BP	178 ± 3	178 ± 3	178 ± 3
	PP	188 ± 11	154 ± 11	179 ± 12
			$P < 0.01$	$P < 0.05$
After atropine plus guanethidine	BP	178 ± 3	178 ± 3	178 ± 3
	PP	188 ± 12	149 ± 9	176 ± 12
			$P < 0.005$	$P < 0.05$

Each value represents mean ± s.e. of results from five animals; it is compared with the corresponding value at time 0 by *t* test for paired samples. BP = blood pressure; PP = perfusion pressure in mmHg.

of noradrenaline and sodium nitrite, proves that the vascular system was reactive following these pharmacological blockades. Furthermore, that the blockade of adrenergic discharge induced by guanethidine treatment was genuinely effective is demonstrated by the lack of change in perfusion and systemic pressure following carotid artery occlusion. We have already demonstrated the capacity of guanethidine to block the sympathetic discharge in the dog (Rengo, De Caprio, Saccà, Trimarco, Perez, Chiariello & Condorelli, 1975a). In this animal, guanethidine is able to reverse the vasoconstriction induced by stimulation of the lumbar sympathetic chain to vasodilatation.

Our results, obtained with the use of [^{14}C]-histamine, confirm the results of Tuttle (1967) and Brody (1968) that radioactive histamine release from

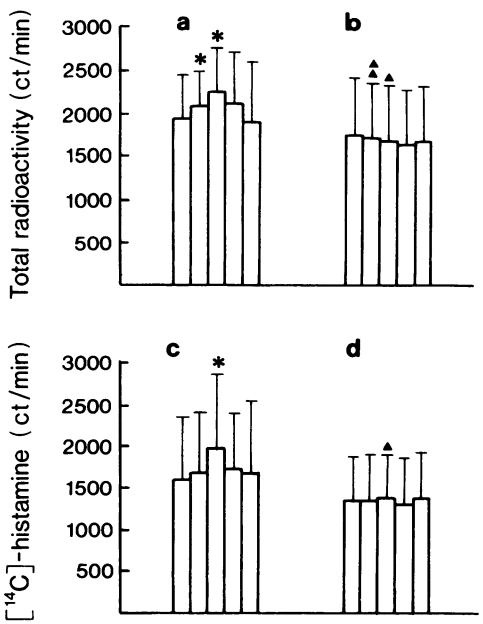

Figure 2 Changes in total and [^{14}C]-histamine radioactivity of the venous blood effluent from the perfused gracilis following the intravenous administration of noradrenaline in basal state (a and c) and after intravenous administration of guanethidine (b and d). Each column shows the radioactivity of venous blood samples collected for 30 s; the first before, the others after the beginning of the reflex vasodilatation. $n = 5$.
*$P < 0.05$ when statistical comparison is made between base and each bar using *t* test for paired samples.
▲$P < 0.05$ and ▲▲$P < 0.01$ when statistical comparison is made between corresponding times before and after intravenous administration of guanethidine by covariance analysis.

Table 2 Changes in total radioactivity (ct/min) of the venous blood effluent from the perfused gracilis muscle of the dog following two intravenous injections of noradrenaline at 30 min intervals

Samples	*1*	*2*	*3*	*4*	*5*
First noradrenaline injection	1591 ± 494 NS	1969 ± 536 NS	1795 ± 473 NS	1609 ± 490 NS	1438 ± 419 NS
Second noradrenaline injection	1273 ± 371	1603 ± 496	1591 ± 382	1413 ± 400	1120 ± 320

Each value represents mean ± s.e. of five experiments. Each sample is collected for 30 s; the first sample before the drug injection, the others after the beginning of the reflex vasodilatation. Statistical comparison is made between corresponding samples of the two stimulations using covariance analysis.

Table 3 Effects of intravenous noradrenaline on (A) systemic and perfusion pressure and (B) total radioactivity (ct/min) of the venous blood effluent from the perfused gracilis muscle before and after selective baroreceptor denervation

A	*Treatment*		*Seconds after drug injection* *0*	*36*	*72*
	Noradrenaline alone	BP	90 ± 8	104 ± 9 $P < 0.05$	94 ± 12 NS
		PP	111 ± 15	89 ± 15 $P < 0.001$	111 ± 15 NS
	After selective baroreceptor denervation	BP	93 ± 10	110 ± 8 $P < 0.005$	90 ± 7 NS
		PP	99 ± 3	95 ± 4 NS	91 ± 8 NS

B	*Samples*	*1*	*2*	*3*	*4*	*5*
	Noradrenaline alone	1050 ± 147	1868 ± 110 $P < 0.01$	1492 ± 72 $P < 0.01$	1214 ± 82 NS	1233 ± 137 NS
	After selective baroreceptor denervation	930 ± 78	924 ± 72 NS	916 ± 58 NS	938 ± 72 NS	941 ± 83 NS

BP = blood pressure; PP = perfusion pressure in mmHg.
Each value represents mean ± s.e. of five experiments. Each sample is collected for 30 s, the first before, the others after the drug injection.
Statistical comparison is made between values at 0 time and each following time using the paired *t* test.

the gracilis muscle increases during reflex vasodilatation. The complete disappearance of the histaminergic component after guanethidine treatment suggests that guanethidine not only inhibits the sympathetic discharge but also abolishes the histaminergic component. This result is of interest because the ability to prevent histamine release has been previously observed only for α-adrenoceptor blocking agents such as phentolamine (Boerth, Ryan & Brody, 1970) and phenoxybenzamine (Heitz & Brody, 1975).

The mechanism underlying histamine release during reflex vasodilatation is not completely clear, Ryan & Brody (1972), in the light of the results obtained by the chronic denervation of the dog gracilis muscle, suggested that either the postganglionic adrenergic fibres innervate the vascular smooth muscle as well as a separate non neural cell from which histamine could be liberated, or that two separate adrenergic fibres exist: one innervating vascular smooth muscle causing vasoconstriction, and a second innervating a histamine containing cell. These hypotheses would be in agreement with the observation of Graham & Lioy (1973) who demonstrated the presence in the lumbar ventral roots of the preganglionic segment of a histaminergic pathway affecting vascular resistance in the hind limb of the dog. These authors suggest, therefore, that the preganglionic segment of this histaminergic pathway runs isolated in the ventral roots from L5 to L7; the postganglionic fibres would then presumably pass into the sympathetic chain together with the adrenergic and cholinergic fibres. Such a view has been also confirmed by recent studies

of Heitz & Brody (1975) on the genesis of the vasodilatation occurring when stimulation of the distal ending of the cut sympathetic chain was terminated.

However, the observation that guanethidine-treatment abolishes histamine release during reflex vasodilatation in the perfused region certainly does not elucidate definitely the mechanisms underlying this phenomenon. In accordance with the hypothesis of Beck, Pollard, Spalding & Wise (1971) that the tonic release of noradrenaline would stabilize the membrane of the histamine containing cell and prevent the liberation of histamine, it might be suggested that the enhanced release of histamine during reflex vasodilatation depends on the withdrawal of the sympathetic discharge. Therefore, guanethidine would supress both the passive component of the reflex vasodilatation and at the same time the release of histamine through the same mechanism, consisting of the abolition of the sympathetic vasoconstrictor tone. Such an interpretation would also be in agreement with the results of Weaver & Gebber (1974) about the lack of excitation of a dilator pathway, running in the sympathetic chain, during reflex vasodilatation. In fact, according to Beck *et al.*'s definition (1971), the histaminergic component of this haemodynamic phenomenon should be considered active only because it takes place through the release of a vasodilator substance and not on account of the electrical activation of a vasodilator nervous pathway.

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