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THE CHOLINERGIC COMPONENT IN THE REFLEX VASODILATATION ELICITED BY STIMULATION OF THE DEPRESSOR NERVES IN THE RABBIT

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1 The effects of the stimulation of the cephalic endings of the depressor nerve on the resistance in the perfused hindlimb were studied in the rabbit.

2 The vasodilatation thus elicited in the perfused hindlimb was reduced either by administration of guanethidine or by sympathectomy and abolished by subsequent treatment with atropine.

3 These data confirm the existence of two components in the genesis of the reflex vasodilatation: a passive component, due to the inhibition of sympathetic discharge, and an active component which in the rabbit is cholinergic in nature.

Introduction

Many investigators conclude that reflex vasodilatation results, at least partly, from an active mechanism since the magnitude of reflex vasodilatation usually exceeds the magnitude of dilatation produced by sympathectomy (Beck, 1961; Beck & Brody, 1961; Sakuma & Beck, 1961). There is a controversy regarding the nature of this active component in the dog. Some investigators suggest it is histaminergic (Beck & Brody, 1961; Beck, 1965; Tuttle, 1965; Brody, 1966; Wellens, 1968) and others suggest it is cholinergic (Rengo, Chiariello, De Caprio, Saccà, Trimarco, Perez & Condorelli, 1975). A histaminergically mediated reflex dilatation has also been observed in the cat (Tuttle, 1967), the rat (Tobia, Myia & Bousquet, 1968) and more recently in the monkey (Levin, Bartlett & Beck, 1968).

The participation of the cholinergic system in the genesis of the reflex has long been denied. However, Takeuchi & Manning (1971; 1973) recently demonstrated the existence of a cholinergic component in the genesis of reflex dilatation in the cat during sustained baroreceptor stimulation with high levels of carotid sinus perfusion pressure. Furthermore, Zanchetti & Ellison (1973) showed the possibility of a muscular cholinergic vasodilatation in the cat.

In this study we wished to demonstrate the participation of the cholinergic system in the reflex vasodilatation elicited in the hindlimb of the rabbit by stimulation of the depressor nerves, which in this animal are anatomically separate from the vagosympathetic trunks.

Methods

Male rabbits, weighing 2–3 kg, were anaesthetized with sodium thiopentone (40 mg/kg i.v.); additional doses of 6 mg/kg were given as required. The trachea was intubated and artificial ventilation was performed with O_2 at a rate of 30–36 cycles per minute.

The hindlimb was perfused at constant flow with blood of the same animal by means of a peristaltic pump (Sigmamotor T6); for this purpose, after administration of heparin (5 mg/kg) by intravenous injection, a polyethylene catheter was introduced through the external iliac artery into the abdominal aorta and the blood was pumped into the femoral artery of the same side. Under these conditions, changes in vascular tone of the perfused hindlimb were reflected by proportional changes in perfusion pressure. The rabbits were paralysed with gallamine triethiodide (3 mg/kg i.v.).

At the beginning of each experiment, blood flow was adjusted to give a perfusion pressure approximately equal to systemic blood pressure and left unchanged throughout the course of the experiment. A thermoregulated delay loop was introduced into the perfusion system so that arterial blood adrenaline arrived at the peripheral muscle after the completion of the haemodynamic response. Perfusion pressure was measured from a T connector interposed between the pump and the hindlimb, and the arterial blood pressure was recorded from a catheter placed in the thoracic aorta via the contralateral femoral artery. Battaglia-Rangoni multichannel polygraph and pressure transducers were used.

The depressor nerves and vagosympathetic trunks

were exposed through an incision in the middle of the neck and then cut. A pair of Ag-AgCl electrodes was placed on the cephalic end of the depressor nerves, covered with mineral oil, and connected to an electrical stimulator (Nihon-Kohden MS 3). Only one trunk was stimulated in each experiment. The stimulation was performed with rectangular waves of 5 V, of 0.2 ms duration, and at a frequency of 20 Hz. The duration of the stimulation was 15 seconds.

In some experiments the sympathetic chain was cut at the lumbar level; in other experiments the sympathetic outflow was blocked by the intravenous administration of guanethidine (8 mg/kg). The effectiveness of the surgical or pharmacological sympathectomy was proved by the lack of change in perfusion pressure following the occlusion of the carotid arteries. The cholinergic blockade was obtained by intra-arterial administration of atropine (0.5 mg/kg). To confirm that the vascular system was reactive following pharmacological blockade induced by surgical sympathectomy or guanethidine and by atropine, the haemodynamic response to intra-arterial administration of sodium nitrite and histamine were evaluated, before and after these treatments, in five experiments.

To exclude the possibility that atropine administration could be blocking nervous transmission at a central, spinal or ganglionic level, five experiments were performed in which the reflex response was evoked by stimulation of the depressor nerve before and after intra-arterial administration of atropine (0.5 mg/kg).

The following drugs were used: sodium thiopentone (Farmotal, Farmitalia); heparin (Liquemin, Roche); guanethidine (Ismelin, Ciba); atropine sulphate (Lancellotti); histamine (Roche) (2 μg i.a.).

Statistical analysis was conducted by a paired t test (Snedecor & Cochran, 1967). All data are presented as mean ± s.e.

Results

Stimulation of the cephalic end of the depressor nerves in the control rabbits elicited a decrease in systemic blood pressure and a reflex vasodilatation in the perfused hindlimb.

Table 1 Effects of stimulation of the depressor nerves on blood pressure and perfusion pressure in hindlimb of rabbit

Treatment		*Seconds after initiation of nerve stimulation*		
		0	*24*	*48*
Stimulation alone	BP	104 ± 11	76 ± 11 $P<0.001$	97 ± 10 NS
	PP	117 ± 20	93 ± 14 $P<0.05$	107 ± 17 NS
After guanethidine (i.v.)	BP	101 ± 10	89 ± 10 $P<0.01$	95 ± 11 NS
	PP	109 ± 12	100 ± 10 $P<0.01$	109 ± 12 NS
After guanethidine plus atropine	BP	95 ± 12	88 ± 12 $P<0.001$	94 ± 11 NS
	PP	102 ± 9	102 ± 9 NS	102 ± 9 NS
Stimulation alone	BP	110 ± 8	71 ± 11 $P<0.001$	97 ± 6 NS
	PP	102 ± 12	83 ± 11 $P<0.01$	100 ± 11 NS
After sympathectomy	BP	99 ± 10	83 ± 15 $P<0.02$	100 ± 12 NS
	PP	106 ± 14	89 ± 13 $P<0.001$	102 ± 12 NS
After sympathectomy plus atropine	BP	99 ± 10	91 ± 10 $P<0.02$	85 ± 7 NS
	PP	101 ± 7	101 ± 7 NS	101 ± 7 NS

Each value represents mean ± s.e. of five animals; it is compared with the corresponding value at time zero. BP = blood pressure; PP = perfusion pressure in mmHg.

After guanethidine pretreatment or surgical sympathectomy the vasodilatation evoked by stimulation of the depressor nerve was reduced. This haemodynamic response was completely abolished by the intra-arterial administration of atropine while the behaviour of the systemic blood pressure was not significantly modified (Table 1).

On the other hand, guanethidine, sympathectomy and atropine produced no modification of the response to the intra-arterial administration of histamine and sodium nitrite (Tables 2 and 3).

Finally, the results summarized in Table 4 show that atropine alone did not abolish the reflex vasodilatation.

Discussion

Our results confirm that the reflex vasodilatation consists of two components: one passive and the other active.

The first component, that results from a decrease in sympathetic vasoconstrictor discharge, is abolished by surgical or chemical sympathectomy induced by

Table 2 Effects of intra-arterial histamine on blood pressure and perfusion pressure in hindlimb of rabbit

Treatment		*Seconds after histamine injection*		
		0	*24*	*48*
Histamine alone	BP	81 ± 4	81 ± 4	81 ± 4
	PP	90 ± 13	113 ± 4	108 ± 3
			$P<0.005$	$P<0.001$
After guanethidine	BP	77 ± 2	77 ± 2	77 ± 2
	PP	94 ± 2	118 ± 3	111 ± 3
			$P<0.005$	$P<0.01$
After guanethidine plus sympathectomy	BP	75 ± 2	75 ± 2	75 ± 2
	PP	90 ± 4	112 ± 3	107 ± 3
			$P<0.001$	$P<0.001$
After guanethidine plus sympathectomy and atropine (i.a.)	BP	75 ± 3	75 ± 3	75 ± 3
	PP	88 ± 3	112 ± 4	105 ± 5
			$P<0.005$	$P<0.005$

Each value represents mean ± s.e. of five animals; it is compared with the corresponding value at time zero. BP = blood pressure; PP = perfusion pressure in mmHg.

Table 3 Effects of intra-arterial injection of sodium nitrite on blood pressure and perfusion pressure in rabbit hindlimb

Treatment		*Seconds after sodium nitrite injection*		
		0	*24*	*48*
Sodium nitrite alone	BP	81 ± 4	81 ± 4	81 ± 4
	PP	87 ± 8	68 ± 8	74 ± 10
			$P<0.005$	$P<0.01$
After guanethidine (i.v.)	BP	78 ± 3	78 ± 3	78 ± 3
	PP	96 ± 4	82 ± 4	81 ± 5
			$P<0.001$	$P<0.001$
After guanethidine plus sympathectomy	BP	75 ± 3	75 ± 3	75 ± 3
	PP	92 ± 4	79 ± 4	79 ± 4
			$P<0.001$	$P<0.005$
After guanethidine plus sympathectomy and atropine (i.v.)	BP	75 ± 3	75 ± 3	75 ± 3
	PP	88 ± 4	73 ± 4	77 ± 3
			$P<0.05$	$P<0.05$

Each value represents mean ± s.e. of five animals; it is compared with the corresponding value at time zero. BP = blood pressure; PP = perfusion pressure in mmHg.

Table 4 Effects of stimulation of the depressor nerves on blood pressure and perfusion pressure in hindlimb of rabbit

Treatment		*Seconds after start of nerve stimulation*		
		0	*24*	*48*
Stimulation of depressor nerves	BP	98 ± 5	81 ± 5 $P < 0.005$	92 ± 4 $P < 0.05$
	PP	100 ± 6	67 ± 6 $P < 0.005$	90 ± 6 $P < 0.05$
Stimulation of depressor nerves after atropine	BP	97 ± 7	82 ± 7 $P < 0.01$	90 ± 7 NS
	PP	98 ± 6	79 ± 6 $P < 0.01$	94 ± 7 NS

Each value represents mean ± s.e. of five animals; it is compared with the corresponding value at time zero. BP = blood pressure; PP = perfusion pressure in mmHg.

guanethidine. That the blockade of adrenergic discharge induced by guanethidine treatment was genuinely effective is demonstrated by the lack of change in perfusion pressure following section of the lumbar sympathetic chain in guanethidine-treated rabbits. On the other hand, we have demonstrated the capacity of this drug to block the sympathetic discharge in the dog (Rengo, De Caprio, Saccà, Trimarco, Perez, Chiariello & Condorelli, 1976). In this animal, guanethidine is able to reverse the vasoconstriction induced by the electrostimulation of the lumbar sympathetic chain to vasodilatation.

The active component in the rabbit is cholinergic in nature because it is abolished by atropine. This finding is very interesting since it confirms the data of Takeuchi & Manning (1971; 1973) about the existence of a cholinergic component in the genesis of the reflex dilatation, which has long been denied.

The observation that atropine alone was not able to abolish the reflex completely rules out the possibility that the dose of atropine here employed could have exerted a muscarinic block at some central or ganglionic level.

Furthermore, the observation that neither surgical sympathectomy nor administration of guanethidine or atropine induces any modification of the haemodynamic response to the intra-arterial injection of histamine and sodium nitrite proved that the vascular system was reactive following surgical or pharmacological denervation.

Regarding the neural pathway of the cholinergic innervation which mediates the active reflex vasodilatation in the rabbit, our results obtained in sympathectomized animals suggest that cholinergic fibres are distributed to the periphery through pathways other than the classical sympathetic outflow, such as the somatic nerves.

The lack of a histaminergic component in the rabbit is not in disagreement with the data of the authors who observed a histaminergically mediated reflex dilatation in several other species (Beck, 1965; Tuttle, 1965; Brody, 1966; Tuttle, 1967; Brody, 1968; Wellens, 1968) since histamine produces a hypertensive effect in the rabbit (Goth, 1968).

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References

BECK, L. (1961). Active dilatation in the innervated perfused hindleg of the dog. *Am. J. Physiol.*, **201**, 123–128.

BECK, L. & BRODY, M.J. (1961). The physiology of vasodilatation. *Angiology*, **12**, 202–221.

BECK, L. (1965). Histamine as the potential mediator of active reflex vasodilatation. *Fed. Proc.*, **24**, 1298–1310.

BRODY, M.J. (1966). Neurohumoral mediation of active reflex vasodilatation. *Fed. Proc.*, **25**, 1583–1592.

BRODY, M.J. (1968). Mechanisms of pharmacologic blockade of reflex vasodilatation. *Fed. Proc.*, **27**, 756.

GOTH, A. (1968). *Medical pharmacology*, Saint Louis: The C.V. Mosby Company.

LEVIN, J., BARTLETT, J.D. Jr & BECK, L. (1968). Active reflex vasodilatation induced by intravenous epinephrine or norepinephrine in primates. *J. Pharmac. exp. Ther.*, **161**, 262–270.

RENGO, F. CHIARIELLO, M., DE CAPRIO, L., SACCÀ, L., TRIMARCO, B., PEREZ, G. & CONDORELLI, M. (1975). Studies on the hemodynamic changes induced by electrical stimulation of the vago-sympathetic trunks in the dog. *Cardiology*, **60**, 98–112.

RENGO, F., DE CAPRIO, L., SACCÀ, L., TRIMARCO, B., PEREZ, G., CHIARIELLO, M. & CONDORELLI, M. (1976). Studies on the nature of vasodilator fibers running in the lumbar sympathetic chain in the dog. *Pharmacology*, **13**, 539–548.

SAKUMA, A. & BECK, L. (1961). Pharmacological evidence for active reflex vasodilatation. *Am. J. Physiol.*, **201**, 129–133.

SNEDECOR, G.W. & COCHRAN, W.G. (1967). *Statistical methods*, Iowa: The Iowa State University Press.

TAKEUCHI, T. & MANNING, J.W. (1971). Muscle cholinergic dilators in the sinus baroreceptor response in cats. *Circulation Res.*, **29**, 350–357.

TAKEUCHI, T. & MANNING, J.W. (1973). Hypothalamic mediation of sinus baroreceptor evoked muscle cholinergic vasodilator response. *Am. J. Physiol.*, **224**, 1280–1287.

TOBIA, A.J., MYIA, T.S. & BOUSQUET, W.F. (1968). Reflex vasodilatation in the perfused hindquarters of the rat. *Pharmacologist*, **10**, 219.

TUTTLE, R.S. (1965). Relationship between blood histamine and centrally evoked hypotensive response. *Am. J. Physiol.*, **209**, 745–750.

TUTTLE, R.S. (1967). Physiological release of histamine ^{14}C in the pyramidal cat. *Am. J. Physiol.*, **213**, 620–624.

WELLENS, D. (1968). Peripheral inhibition of reflexogenic vasodilatation induced by Veratridine. *Arch. Int. Pharmcodyn.*, **172**, 1–12.

ZANCHETTI, A. & ELLISON, G.D. (1973). Diffuse and specific activation of sympathetic cholinergic fibers of the cat. *Am. J. Physiol.*, **225**, 142–149.

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