Short communications

Peripheral cardiovascular effects, in the pithed rat, of compounds used in the treatment of hypertension

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A method for stimulating discrete segments of the spinal autonomic outflow in the pithed rat was found suitable for differentiating between the group of centrally acting antihypertensive agents α-methyldopa, clonidine and iproniazid and other antihypertensive compounds which additionally have peripheral vascular effects mediated via the peripheral sympathetic nerves.

A method for stimulation of the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat (Gillespie & Muir, 1967) was recently refined to permit the stimulation of discrete segments of the spinal autonomic outflow (Gillespie, Maclaren & Pollock, 1970). This method provides a suitable means whereby peripheral actions of agents may be studied. It seemed possible that agents with primarily central actions on blood pressure could be easily distinguished from peripherally acting agents, since only the latter would be expected to affect blood pressure and/or the blood pressure response to electrical stimulation. This possibility was tested with a number of drugs used in the treatment of hypertension.

Methods.—Male rats (200–275 g) were pithed as described by Gillespie *et al.* (1970). The stimulating electrode was inserted via the orbit and *foramen magnum* into the spinal cord to a distance of 8 cm and the spinal cord was stimulated at vertebral level T7–9. This level was chosen in order to obtain maximal blood pressure responses and minimal effects of other responses. In some experiments rats were pithed to vertebral level of T9 only, but the effects of drugs were the same as those observed in completely pithed preparations. Pulses (frequency 10 Hz, duration 1·0 ms) were given for 30 s at five times threshold voltage. This procedure was repeated every five min; recovery from each stimulation was complete within four minutes. Blood pressure was measured with a Statham P23Db transducer from the carotid artery and recorded on a Philips cardiopan 3R recorder. All rats were pretreated with tubocurarine (1 mg/kg i.v.) and maintained by artificial respiration.

The drugs used were α-methyldopa (Aldomet; Merck, Sharp and Dohme), clonidine hydrochloride (Catapres; Boehringer), chlorpromazine hydrochloride (Rhône-Poulenc), diazoxide (Schering), dihydroergotamine (Merck), guanethidine sulphate (CIBA), hydrochlorothiazide (Merck, Sharp and Dohme), pargyline phosphate (Abbott), sodium phenobarbitone (Abbott), reserpine (Merck), sodium thiocyanate (Baker), tiprenolol hydrochloride (Philips-Duphar, not yet available in U.K.), trimethaphan camphor sulphonate (Arfonad; Roche), (+)-tubocurarine chloride (Nourypharma). All drug solutions were freshly prepared each day and were injected into the femoral vein. Drugs were injected (0·25 ml/250 g weight of rat) 1 min before the next electrical stimulation, in increasing doses with a dose increment of 3. Only one agent was tested per rat. Doses refer to the salts.

Results.—Control rats, in which the resting blood pressure was 64±3 mmHg, responded to sympathetic stimulation with an increase in blood pressure of 94±22 mmHg (±S.D.; n=77). The magnitude of this response was remarkably constant over a period of up to 4 h in any one rat. Control injections of saline in the same volume as for drug additions had little effect upon the blood pressure or response to sympathetic stimulation. The effects of various antihypertensive compounds on blood pressure, on the magnitude and maximal level of the response upon sympathetic stimulation were measured (Table 1). Doses of the antihypertensive agents were chosen on the basis of blood pressure response in the unpithed rat anaesthetized with sodium pentobarbitone. For this purpose the compounds were administered in increasing dose levels—dose increment 3. The intravenous dose that produced, in the rat, within 20 min, at least a 15 mmHg fall in pressure in 3 to 6 animals, was taken as the lowest dose level in the pithed

rat experiments. The maximum effect of drugs on sympathetic stimulation was usually observed within 6 min of drug administration, but lower doses were effective only after longer periods (up to 90 minutes). The results with the lowest dose were scored as effective (E) or not effective (N) depending upon whether the parameter changed by more than an arbitrary amount (Table 1).

TABLE 1. *Effects of various agents on blood pressure and blood pressure responses following sympathetic stimulation in the pithed rat*[1]

Compound	Dose (mg/kg)	Maximal effect on blood pressure[2] (Average in mmHg)		Decrease of the response upon sympathetic stimulation[3] (Average in mmHg)		Decrease of the maximal level upon stimulation[3] (Average in mmHg)	
		Lowest dose	Highest dose	Lowest dose	Highest dose	Lowest dose	Highest dose
Saline	0·25 ml/250 g		−3		2		2
α-Methyldopa[4]	30·0	0 (N)		3 (N)		3 (N)	
	100·0		+17		1		1
Chlorpromazine	0·1	−8 (E)		47 (E)		37 (E)	
	3·0		−15		72		87
Clonidine	0·03	0 (N)		3 (N)		3 (N)	
	1·0		+40		63		41
Diazoxide	1·0	−10 (E)		0 (N)		10 (E)	
	30·0		−15		53		69
Dihydroergotamine	0·01	−4 (N)		14 (E)		20 (E)	
	3·0		+23		107		80
Guanethidine	1·0	−19 (E)		40 (E)		60 (E)	
	10·0		−17		98		110
Hydrochlorothiazide[5]	10·0		+5		12		18
Iproniazid	10·0	−3 (N)		2 (N)		5 (N)	
	30·0		+8		24		8
Mecamylamine	0·3	−5 (N)		47 (E)		51 (E)	
	1·0		−15		74		94
Pargyline	10·0	+7 (E)		37 (E)		33 (E)	
	30·0		−17		32		40
Phenobarbitone[6]	30·0	−25 (E)		32 (E)		25 (E)	
Reserpine	0·3	−15 (E)		3 (N)		11 (E)	
	10·0		−21		30		49
Thiocyanate[7]	10·0	+8 (E)		7 (N)		0 (N)	
	100·0		+18		28		20
Tiprenolol	0·1	−10 (E)		+12 (E)		3 (N)	
	10·0		−44		77		93
Trimethaphan	0·3	−16 (E)		36 (E)		50 (E)	
	3·0		−11		78		103

[1] n=4–7 for each agent.

[2] + =increase of blood pressure.
− =decrease of blood pressure. This column refers to changes in blood pressure elicited by the drug itself. (E)=a change in blood pressure (+or−) by more than 6 mmHg; (N)=less than 6 mmHg change.

[3] (E)=a change in the magnitude of the blood pressure response (or in the maximal resting level of blood pressure) by 10 or more mmHg observed upon stimulation; (N)=a change of less than 10 mmHg.

[4] Hypotensive responses to α-methyldopa in unpithed anaesthetized rats were studied for a 2 h period after drug administration. The responses in the pithed rat were also studied for at least two hours.

[5] No effects on blood pressure in unpithed anaesthetized rats with hydrochlorothiazide (to 30 mg/kg) were observed within 30 min of drug administration.

[6] Only one dose of phenobarbitone is reported since 10 mg/kg was ineffective in lowering the blood pressure of unpithed rats by 15 mmHg and a dose of 100 mg/kg was lethal in all three pithed rats studied.

[7] All doses of thiocyanate studied (10, 30, 100 mg/kg) elicited no effect or a blood pressure rise in unpithed rats.

Discussion.—The agents studied were placed in various categories for the purposes of discussion.

Autonomic agents

Drugs acting at neuronal sites	(Guanethidine, reserpine)
α-Adrenoceptor antagonists	(Chlorpromazine, dihydroergotamine)
β-Adrenoceptor antagonists	(Tiprenolol)
Ganglion blocking agents	(Mecamylamine, trimethaphan)
Monoamine oxidase inhibitors	(Iproniazid, pargyline)
Non-catecholamine sympathomimetic agents	(Clonidine)

Agents affecting the central nervous system

Agents with direct actions on the vasomotor centre	(α-methyldopa, clonidine)
Antidepressants	(Iproniazid)
Hypnotics	(Phenobarbitone)
Neuroleptics	(Chlorpromazine, reserpine)

Miscellaneous agents

Diuretics	(Hydrochlorothiazide)
Smooth muscle relaxants	(Diazoxide, sodium thiocyanate)

α-Methyldopa, clonidine and iproniazid at the lowest dose did not influence the three parameters used as an indication of peripheral circulatory function. This suggests that their hypotensive effect in anaesthetized rats was mediated through the central nervous system. Three other compounds with a known central activity, chlorpromazine, reserpine and phenobarbitone, clearly affected the peripheral parameters studied. For chlorpromazine and reserpine these peripheral effects upon sympathetic nerve function are well known. Phenobarbitone may have caused general cellular depression since it was only studied at a rather high dose.

At higher doses most agents, including clonidine and iproniazid had moderate to strong effects on the parameters measured. This suggests that antihypertensive agents that have centrally mediated effects may exert at least some peripheral actions at higher doses.

This technique seems well suited for studying peripheral circulatory effects of antihypertensive drugs that have a fairly rapid onset of action. With this preparation, agents such as α-methyldopa, clonidine and iproniazid which affect blood pressure primarily by a central action can be differentiated from other drugs which have peripheral vascular effects mediated additionally via the peripheral sympathetic nerves.

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