Short communications

Augmentation of drug-induced blood pressure increases in rats by amobarbital

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The effects of amobarbital anaesthesia on blood pressure responses to angiotensin and noradrenaline were determined in rats with chronically implanted venous and aortic catheters. Responses were tested twice on the same day in each rat, first in the conscious state and then during anaesthesia. Blood pressure rises produced by noradrenaline and angiotensin were larger when the rats were anaesthetized with amobarbital than when they were conscious. This enhancement was not characteristic of general anaesthesia since urethane, instead of enhancing, depressed the pressor responses.

Blockade of acetylcholine receptors with atropine also increased the response to pressor drugs. However, amobarbital in anaesthetic doses did not affect the response to acetylcholine, indicating that removal of compensatory parasympathetic reflexes was not a major cause of its enhancing effect. The effects of amobarbital on arterial pressure and heart rate were similar to those of the ganglion blocking drug pentolinium. When pressor responses were increased by amobarbital, autonomic ganglia were depressed as indicated by the loss of the response to nicotine. These findings suggest that amobarbital enhances blood pressure responses to noradrenaline and angiotensin by blocking autonomic ganglia.

Rats anaesthetized with urethane are generally assumed to be less sensitive to angiotensin than those anaesthetized with barbiturates (Page & McCubbin, 1968), but the mechanism of action is unknown. The effects of angiotensin and noradrenaline were therefore studied in rats with chronically implanted vascular catheters from which cardiovascular responses to intravenously injected drugs could be recorded, first, in the conscious state and then during anaesthesia. Subsequent experiments were done to determine whether blockade of acetylcholine receptors with atropine or ganglion blockade accounted for the enhancement by amobarbital of the pressor responses to noradrenaline and angiotensin.

Methods.—Female Wistar rats (250–300 g) were anaesthetized with ether; one catheter was inserted into the lower abdominal aorta for recording arterial pressure and another one into the right jugular vein for the injection of drugs. The technique was as described earlier (Buñag, 1971; Buñag, McCubbin & Page, 1971). Two to three days were allowed for recovery from surgery.

The rats were kept in open-topped cages during all tests by using the harness-and-swivel arrangement described previously (Buñag, 1971). The aortic pressure was recorded by connecting the indwelling aortic catheter to a Statham P23Gb pressure transducer located about 36 cm above each cage. For simultaneous recording of heart rate, the arterial pulse signals from the transducer were fed into a biotachometer (Brush Instruments, Cleveland, Ohio). All drugs other than the anaesthetics were injected through tygon tubing taped to the spring connector of the swivel and connected to the indwelling jugular catheter. The total dead space of the system for intravenous injections, including the indwelling catheter, was 0·25–0·3 ml.

Blood pressure responses were tested in each rat first while it was conscious and then after it was anaesthetized with either amobarbital sodium (Amytal, 8 mg/100 g body weight, i.p.) or urethane (100 mg/100 g body weight, i.p.). In some experiments the acetylcholine receptors were blocked with atropine sulphate (0·5 mg/rat i.v.) and in others the ganglion blocking drug pentolinium tartrate (Ansolysen, 1·0 mg/rat i.v.) was used. Various doses of angiotensin (5–50 ng) and noradrenaline (50–500 ng) were injected intravenously while aortic pressure was recorded continuously. Blockade of acetylcholine receptors with atropine was considered complete when depressor responses to acetylcholine (400 ng) were abolished. Abolition of pressor responses to nicotine (20 μg) was used to gauge the degree of ganglion blockade produced by either amobarbital or pentolinium. Except when

indicated otherwise, all doses are expressed as the amount of drug injected per 100 g body weight.

Results.—Blood pressure responses to noradrenaline and angiotensin vary spontaneously in anaesthetized animals (Buñag, McCubbin & Page, 1968) and even more in conscious rats. Since variability increased in each conscious rat with the number of injections made, only two doses of angiotensin and noradrenaline were used per rat, and these selected test doses were injected alternately at 6–8 min intervals. Injection of each dose was repeated until at least two identical responses were recorded. Each drug was injected in a volume of 0·1 ml followed by 0·4 ml of 0·9% sodium chloride. Control injections of 0·5 ml saline alone were routinely given and experiments in which these produced significant pressor responses (<10 mmHg) were discarded. By keeping the conditions during the tests as constant as possible, reproducible pressor responses to both noradrenaline and angiotensin could be obtained. Reflex bradycardia usually occurred at the same time as the pressor response to either drug. Since only two test doses were used for each drug, full dose-response curves could not be constructed, but, within the dose range employed, the magnitude of the pressor response and its associated reflex bradycardia were usually dose-dependent.

After recording the pressor responses to noradrenaline and angiotensin in 15 conscious rats, they were anaesthetized with amobarbital. When their blood pressures had stabilized 15–30 min later, the pressor responses to noradrenaline and angiotensin were tested a second time. Amobarbital anaesthesia not only stabilized but also lowered the aortic pressure from 109±3 (S.E.M.) mmHg in the conscious state to 81±5 mmHg ($P<0.005$) during anaesthesia. The heart rates fell from 452±15 beats/min to 344±14 beats/min ($P<0.005$). Pressor responses to both noradrenaline and angiotensin increased significantly in 11 rats, while they were unaltered in 4 (Table 1A). In rats that did not show augmentation after amobarbital, the initial responses were generally larger. There was no appreciable difference in the enhancement of the responses to the two test drugs. Reflex bradycardia elicited by either drug was always reduced during amobarbital anaesthesia.

Following the same procedures, the pressor responses to angiotensin and noradrenaline were recorded before and after urethane anaesthesia in 7 other conscious rats. Like amobarbital, urethane lowered

TABLE 1. *The effect of anaesthesia and ganglion blockade on responses of blood pressure and heart rate to noradrenaline and angiotensin in the rat*

	Test drug*	Mean pressor response (mmHg) Awake	Mean pressor response (mmHg) Anaesthetized	Reflex bradycardia (heart rate/min) Awake	Reflex bradycardia (heart rate/min) Anaesthetized
A. Effects of amobarbital anaesthesia					
1. Augmented	Noradrenaline	38±3	66±3	−131±15	−25±18
(n=11)	Angiotensin	46±3	67±4	−150±17	−20±14
2. Unaffected	Noradrenaline	43±7	46±7	−116±11	−5±20
(n=4)	Angiotensin	57±4	59±9	−123±17	−19±20
B. Effects of urethane anaesthesia					
(n=7)	Noradrenaline	47±4	26±5	−104±11	−23±14
	Angiotensin	53±2	33±5	−109±9	−8±12
		Awake Before Pent.	Awake After Pent.	Awake Before Pent.	Awake After Pent.
C. Effects of pentolinium					
(n=9)	Nicotine	34±4	1±0·8	−83±29	+5±5
	Noradrenaline	32±2	65±7	−130±27	+3±16
	Angiotensin	35±5	61±7	−89±30	−19±11

* Doses per 100 g body weight are 200 ng for noradrenaline, 20 ng for angiotensin and 20 μg for nicotine. All numbers are average changes±S.E.M. from basal or pre-injection values. Pent.: pentolinium (1·0 mg/rat, i.v.)

the aortic pressure from 115±2 mmHg to 89±10 mmHg ($P<0.025$). The effect of urethane on heart rate was insignificant (conscious state: 432±10 beats/min; during anaesthesia: 405±20 beats/min). In contrast to the enhancement produced by amobarbital, the blood pressure responses to angiotensin and norepinephrine were always reduced during urethane anaesthesia (Table 1B). These results showed that general anaesthesia itself was not responsible for the increase in pressor responses observed in rats anaesthetized with amobarbital.

Compensatory parasympathetic reflexes normally limit the quick rise in arterial pressure produced by noradrenaline or angiotensin. The abolition of such reflexes can thus be expected to enhance responses to any pressor agent, and this may be the mechanism by which amobarbital acts. To study this possibility, blood pressure responses were recorded in 6 conscious rats before and 15–30 min after the injection of atropine sulphate (0·5 mg/rat i.v.). Atropine did not affect mean aortic pressures (112±2 mmHg before, and 109±2 mmHg after atropine injection) but heart rates were accelerated (417±12 beats/min before and 480±14 beats/min, $P<0.005$) after atropine. The effects of atropine on the cardiovascular responses to noradrenaline and angiotensin were similar to those of amobarbital; the pressor responses were enhanced while reflex bradycardia was reduced, presumably due to a suppression of compensatory parasympathetic reflexes. The hypotensive response to an injection of acetylcholine (400 ng) was abolished by atropine but unaffected by amobarbital. Therefore, it seems unlikely that blockade of acetylcholine receptors is responsible for augmentation by amobarbital of the blood pressure responses to angiotensin and norepinephrine.

As it is well established that ganglion blocking drugs enhance blood pressure responses (Page & McCubbin, 1963), it is possible that amobarbital augments responses to angiotensin and noradrenaline by its ganglion blocking activity (Exley, 1954). Accordingly, blood pressure responses were tested in 9 conscious rats before and after an injection of the ganglion blocking drug, pentolinium (1·0 mg/rat i.v.). Pentolinium, like amobarbital, decreased the mean aortic pressure (controls: 112±2 mmHg; after pentolinium: 72±3 mmHg) and the heart rate (control: 431±9 beats/min; after pentolinium: 359±10 beats/min; $P<0.005$). The pressor responses to noradrenaline and angiotensin were invariably increased while those to nicotine (20 μg) were abolished (Table 1C). In 4 other rats anaesthetized with amobarbital, enhancement of pressor responses was accompanied by a loss of the response to nicotine, thus confirming its ganglion blocking action.

Discussion.—Our findings indicate that blood pressure rises produced by noradrenaline and angiotensin are increased by amobarbital anaesthesia. This enhancing effect is not due simply to general anaesthesia, because with urethane blood pressure responses are depressed. When responses to noradrenaline and angiotensin are increased, those to nicotine are abolished while those to acetylcholine are unaffected. This suggests that ganglion blockade by amobarbital accounts for its augmentation of response to pressor drugs.

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