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The evaluation of cardiovascular drugs in the anaesthetized, unrestrained rat

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A method is reported which allows continuous long-term drug administration and simultaneous blood pressure measurement in the unanaesthetized unrestrained rat. The external jugular vein and abdominal aorta were cannulated and the opposite ends of the cannulae were passed subcutaneously and exteriorized at the back of the head. They were then passed through a spring attached at the lower end to the skull and, at the upper end, to a counterweighted cantilever. In rats so prepared, infusion of angiotensin amide 200 ng kg^{-1} min^{-1} caused a rise of blood pressure which lasted the 48 h infusion period. Heart rate decreased initially but recovered within 6 h. Angiotensin amide 30 ng kg^{-1} min^{-1}, infused up to seven days, was without effect on blood pressure or heart rate, and both doses of angiotensin amide failed to alter cardiac catecholamine turnover. Hydralazine, mecamylamine and clonidine reduced blood pressure to 63, 62 and 84% of control respectively while clonidine induced a transient increase before its depressor effect. Heart rate was increased by hydralazine to 138%, and decreased by clonidine to 74% of control, and was unaffected by mecamylamine. The magnitude of pressor response to noradrenaline, tyramine and angiotensin was reduced by hydralazine and increased by mecamylamine. Clonidine increased the pressor response to angiotensin but had no effect on that to noradrenaline or tyramine.

A number of methods for blood pressure measurement in the rat have been described (Weeks & Jones, 1960; Weeks, 1964; Dalton, Touraine & Wilson, 1969; Browning, Ledingham & Pelling, 1970; Van Petten, Evans & Salem 1970; Stanton, 1971; Laffan, Peterson & others, 1972; Bunag, 1973; Buckingham, 1976). Blood pressure measurement directly through an arterial cannula has the advantage that precision and sensitivity are excellent and monitoring continuous. It is usually made by restraining the rat in order to connect the arterial cannula to a transducer and recording apparatus (Weeks & Jones, 1960; Stanton, 1971). Apart from being stressful, such restraint limits the time that measurements can be made. Weeks (1964) used a harness and swivel that allowed either continuous drug infusion or blood pressure measurement in the unrestrained rat. Analogous systems have been reported by Dalton & others (1969) and Laffan & others (1972). None of these methods allows both long term continuous drug administration and simultaneous blood pressure measurement in the unrestrained rat. This has been done in the present study and the method has been used to evaluate several cardiovascular agents.

MATERIALS AND METHODS

Male Sprague-Dawley rats, 325–375 g were prepared according to a modification of the method of Weeks & Jones (1960). The rats were anaesthetized in early experiments with pentobarbitone sodium (Nembutal, Abbott), (50 mg kg^{-1}, i.p.) and in later experiments with ketamine hydrochloride (Ketaject), (120 mg kg^{-1}, i.p. and additional doses of 15 mg kg^{-1}, i.v. every 15 to 20 min, since ketamine increased survival during surgery from 50 to 90% compared with pentobarbitone which caused up to 40 mg Hg decrease in b.p.).

A cannula inserted into the external jugular vein was pushed to within 0·5 cm of the right atrium and ligated. The opposite end was passed subcutaneously and exteriorized midline between the ears. After midline laparotomy, and the clamped abdomial aorta had been punctured with a 26 gauge hypodermic needle, a cannula was inserted retrogradely to below the renal arteries (see Fig. 1a). The cannula was sutured to the psoas muscle at the level of the iliolumbar vessels, and was passed subcutaneously along the back to be exteriorized with the venous cannula. The incision was then closed using subcutaneous stitches. The cannulae were then passed through a spring-cantilever device attached to the skull in the following manner. An incision was made along the midline, and the skull surface cleaned and dried. Two holes were drilled in the parietal plate caudal to the coronal sutures and 2 mm off midline taking care not to puncture the dura (Fig. 2A). Stainless steel sockethead bolts (1–72 × 5/16) were screwed securely into these holes, after which the spring (0·6 cm × 30 cm) was brought into close

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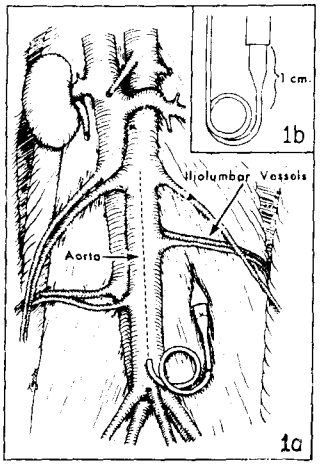

FIG. 1. Placement of the aortic cannula. The cannula was sutured to the psoas muscle and advanced to below the renal arteries (1a). The introduction of a loop in the cannula (1b) polonged its functional life.

apposition to the bolts and skull and glued in place with dental cement (Fig. 2B).

The upper end of the spring was attached to a cantilever arrangement constructed from aluminium channel and sheet metal. All connections between aluminium parts were secured with standard steel bolts, and oversized holes were drilled at joints so that the cantilever moved freely in the vertical axis and the fulcrum support moved freely in the horizontal axis. A three ounce lead weight was attached to the short arm of the cantilever to counterbalance the combined weight of the opposite arm and the spring. The cantilever was 38 cm in length with the fulcrum positioned 8 cm off centre. These dimensions were sufficient to allow the rat full freedom of movement in a cage with a base of 35 × 35 cm.

The cannulae were prepared from polyethylene tubing (PE intramedic tubing; Clay Adams, Parsippany, N.J.) and were modified from those described by Weeks & Jones (1960). One end of a length of PE 50 tubing was pulled out until the diameter of the pulled section approximated that of PE 10 tubing (0·61 mm). This was then cut at an angle to make a sharp tip. No further manipulation was required for the venous cannula but for the aortic cannula the pulled section was bent to form a J or, in later experiments, a complete loop (Fig. 1b) and was fixed with boiling water. The loop is superior to the J because it prolongs the functional life of the cannula by preventing the tip from moving inside the aorta with change in body position (J. R. Weeks, personal communication). Immediately beyond the loop, the PE 50 tubing was fitted into PE 100 tubing and sealed with cyanoacrylate glue (Eastman 910: Eastman Kodak, Rochester, New York). This minimized damping of the pulse pressure amplitude.

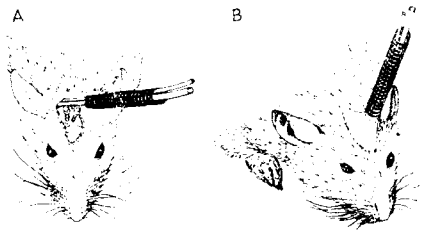

FIG. 2. Attachment of spring enclosing the cannulae to the skull of the rat. Two holes were drilled in the parietal plate into which bolts were screwed (2A). The spring was brought into close apposition to the bolts (2B), and glued in place with dental cement. The upper end of the spring was attached to a cantilever.

During blood pressure measurement, heparinized (10 U ml^{-1}) dextrose 5% solution was infused continuously at 1 ml h^{-1} through the aortic cannula to prevent cell packing in the tip. It did not affect blood pressure. Drugs were made up in this solution and, in some experiments, delivered in a 1 ml volume over 1 min using an infusion pump.

In those experiments where relative cardiac catecholamine turnover was determined, rats received tritiated noradrenaline (^{3}H-NA) (30 μCi kg^{-1}, 3·8 Ci $mmol^{-1}$, i.v.) 30 min before infusion began. After 24 h of infusion, control and drug-treated animals were anaesthetized with pentobarbitone sodium (50 mg kg^{-1}) and the hearts were removed, washed, blotted, and homogenized in ice-chilled 0·4 M perchloric acid using a Sorvall Omni-mixer. After centrifugation the supernatant was adjusted to pH 8·4 and placed on an alumina column. Noradrenaline and ^{3}H-NA were eluted with 0·2 M HCl. One ml of the eluate was assayed by liquid scintillation spectrometry to determine tissue radioactivity and the remainder was assayed spectrofluorometrically (Chang, 1964) to determine content of endogenous noradrenaline.

Drugs used were: hydralazine hydrochloride (Apresoline, Ciba), mecamylamine hydrochloride (Sigma Chemical Co.), clonidine hydrochloride (Catapres, courtesy of Boehringer Ingelheim, Ltd.), (−)-noradrenaline bitartrate (Levophed, Winthrop Laboratories), [^{3}H]noradrenaline (New England Nuclear), angiotensin amide (Hypertensin, Ciba), and tyramine hydrochloride (Sigma Chemical Co.). All drug concentrations were calculated using base weight. Statistical analysis was carried out using the

two-tailed *t*-test for paired or unpaired data and differences at $P < 0.05$ were considered significant.

RESULTS

Prepared rats were used in the evaluation of several vasoactive agents. Long-term drug infusion was carried out using angiotensin amide which at 30 ng kg^{-1} min^{-1} either had no pressor effect or raised the blood pressure less than 5 mm Hg, while at up to 600 ng kg^{-1} min^{-1} it elicited a dose related, sustained increase in blood pressure without the development of tachyphylaxis. An infusion of a pressor dose of 200 ng kg^{-1} min^{-1} was carried out in six rats over 48 h and this increased the blood pressure from 85 ± 9 to 140 ± 7 mm Hg ($P < 0.001$). Heart rate decreased from 332 ±18 to 208 ± 6 ($P < 0.01$). The blood pressure rise lasted throughout the infusion but the heart rate returned to control values in 2 to 6 h. During the infusion all six rats exhibited increasingly severe watery discharge from the nose, slightly bloody tears and diarrhoea and their coats became matted and oily. All symptoms disappeared 24 h after the infusion was replaced by heparinized dextrose 5% solution.

In a second experiment, angiotensin 30 ng kg^{-1} min^{-1} was infused in three rats for four days and in a second group of three rats for seven days. There were no significant changes in blood pressure or heart rate, but both groups exhibited the same symptoms as those seen in the rats on the pressor dose of angiotensin. Their development was slower but ultimately the same level of severity was reached necessitating replacement of the angiotensin solution by heparinized dextrose 5% solution.

To determine the functional life of this rat preparation, infusion and blood pressure measurements were made continuously for three weeks in the experiments described above. It was the functional life of the aortic cannula that determined the time for which the preparation could be used. All 12 cannulae remained functional throughout the first week. However, three failed during the second week and the remainder during the third week. The cannula was considered to have failed when a pulse pressure could not be detected even after injection of solution into it, although fluid could be injected blood could not be withdrawn. Thus, the functional life was one to two weeks.

The effect of angiotensin infusion on the content and turnover of cardiac catecholamines was assessed in rats prepared with venous cannulae. Thirty min after pretreatment with ^{3}H-NA, infusion was begun in rats receiving one of the following treatments: (1) angiotensin amide, 300 ng kg^{-1} min^{-1}, (2) angiotensin amide, 30 ng kg^{-1} min^{-1}, (3) heparinized dextrose 5% solution, (4) hydralazine, 1 mg kg^{-1} (i.v.) immediately before starting an infusion of heparinized dextrose 5% solution. Twenty-four h later, the rats were killed and hearts removed for analysis of content of endogenous noradrenaline and ^{3}H-NA.

The decline of ^{3}H-NA from the rat heart is a reflection of the level of sympathetic tone (Brodie, Costa & others, 1966). Thus, the amount of ^{3}H-NA remaining after 24 h was considered a relative measure of cardiac noradrenaline turnover and the data are shown in Table 1. Infusion of pressor and subpressor doses of angiotensin had no effect on the cardiac content of ^{3}H-NA, but hydralazine 1 mg kg^{-1} reduced the content of ^{3}H-NA to 28% of control. None of the treatments altered content of endogenous noradrenaline. Hydralazine is known to induce tachycardia mediated, in part, by increased sympathetic nerve activity (Brunner, Hedwall & Meier, 1967). Thus, it was expected to reduce ^{3}H-NA content and was included in this study as a validation of the turnover assay.

In another series of experiments, rats prepared with venous and aortic cannulae were used to evaluate the effects of hydralazine, mecamylamine, and clonidine on blood pressure and heart rate, and on the magnitude of pressor response to noradrenaline, tyramine and angiotensin. The effects of the depressor agents on blood pressure and heart rate are shown in Table 2. Hydralazine, mecamylamine and clonidine reduced blood pressure to 63, 62 and

Table 1. *Effect of angiotensin and hydralazine on decline of ^{3}H-NA and on endogenous noradrenaline content in the rat heart.* ^{3}H-NA content is expressed as d min^{-1} g^{-1} tissue and endogenous noradrenaline as μg g^{-1} tissue. Values represent means ± s.e.

Treatment	n	^{3}H-NA content	Endogenous noradrenaline content
Control	7	150234 ± 10708	0·762 ± 0·067
Hydralazine 1 mg kg^{-1}	6	42395 ± 7476*	0·685 ± 0·029
Angiotensin 30 ng kg^{-1} min^{-1}	8	161523 ± 20310	0·778 ± 0·057
Angiotensin 300 ng kg^{-1} min^{-1}	9	149709 ± 14396	0·708 ± 0·049

* $P < 0.01$.

Table 2. *Effect of hydralazine, mecamylamine and clonidine on blood pressure and heart rate.* Blood pressure is expressed as mm Hg and heart rate as beats min^{-1}. Four to six determinations were made and each rat served as its own control. Values represent means ± s.e.

Treatment	B.p.	% cont.	Heart rate	% cont.
Control	92·5 ± 6·0		342 ± 11·0	
Hydralazine (1 mg kg^{-1})	60·0 ± 4·2*	63	471 ± 16·1*	138
Control	100·0 ± 5·2		324 ± 31·3	
Mecamylamine (2·5 mg kg^{-1})	62·2 ± 5·2*	62	375 ± 29·2	N.S.
Control	95·3 ± 4·4		356 ± 13·0	
Clonidine (50 μg kg^{-1})	80·5 ± 5·8*	84	263 ± 9·8*	74

* $P < 0{\cdot}01$.
N.S. = Not significant.

Table 3. *Effect of hydralazine, mecamylamine and clonidine on the magnitude of pressor response to noradrenaline, tyramine and angiotensin.* Values represent the mean increase in blood pressure expressed as mm Hg ± s.e. Five to six determinations were made and each rat served as its own control.

Treatment	Noradrenaline (0·15 μg kg^{-1})	% cont.	Tyramine (150 μg kg^{-1})	% cont.	Angiotensin (0·15 μg kg^{-1})	% cont.
Control	28·7 ±3·9		54·6 ±15·4		32·7 ±4·7	
Hydralazine (1 mg kg^{-1})	12·2 ±2·3**	43	7·4 ±8·1**	14	11·4 ±6·9**	35
Control	36·3 ±5·8		43·6 ±10·5		36·2 ±6·2	
Mecamylamine (2·5 mg kg^{-1})	51·6 ±6·6**	142	73·6 ±4·7**	169	67·0 ±12·6**	185 185
Control	31·6 ±5·5		53·8 ±5·7		32·0 ±3·0	
Clonidine (50 μg kg^{-1})	46·2 ±7	N.S.	59·0 ±4·6	N.S.	55·2 ±6·45*	173

* $P < 0{\cdot}05$.
** $P < 0{\cdot}01$.
N.S. = Not significant.

84% of control respectively. Clonidine induced a transient increase in blood pressure before its depressor effect. Heart rate was increased by hydralazine to 138% and decreased by clonidine to 74% of control, and was unaffected by mecamylamine.

The effect of each of these depressor agents on the size of response to noradrenaline, tyramine and angiotensin is shown in Table 3. Noradrenaline, tyramine and angiotensin were administered sequentially intravenously and the blood pressure response to each measured. Then, either hydralazine, mecamylamine or clonidine was administered and, after blood pressure stabilization, administration of the three pressor agents was repeated. In the presence of hydralazine, the size of pressor response to noradrenaline, tyramine and angiotensin was reduced to 43, 14 and 35% of control, respectively. But mecamylamine increased the response to these agents to 142, 169 and 185%, respectively. The effect of clonidine on the activity of the three pressor agents was not uniform. The response to angiotensin was increased to 173% of control while that to noradrenaline and tyramine was not significantly altered.

DISCUSSION

The unique advantage of the spring cantilever apparatus described lies in the fact that the rat may be left unattended, e.g., overnight while infusion and blood pressure recording are carried out. In addition, the apparatus allows remote intravenous drug injection. These advantages are a result of the spring which blocks the rat's access to the cannulae and possesses sufficient torque to prevent the rat from rotating more than two or three turns in either direction. Thus, the rat can neither chew nor twist the cannulae.

It was found that the functional life span of the aortic cannula determined the time over which the rat could be used for infusion and blood pressure measurement. In these experiments, the aorti cannulae remained functional for one to two weeks. Others (Van Petten & others, 1970; Buckingham, 1976) have reported a similar aortic cannula life span and have attributed cannula failure to the development of a fibrin plug in the cannula tip or to damage of the intimal surface of the arterial wall in the immediate vicinity of the cannula tip.

Angiotensin has been infused over long periods in the dog (McCubbin, DeMoura & others, 1965), rabbit (Dickinson & Yu, 1967), and monkey (Forsyth, Hoffbrand & Melmon, 1971) with no reported effect on the state of health, while we found the rat to deteriorate in health. Furthermore, the symptoms were not related to increased blood pressure since they occurred with both pressor and subpressor doses of angiotensin. Intravenous infusion of subpressor angiotensin in the dog (McCubbin

& others, 1965), rabbit (Dickinson & Yu, 1967), and monkey (Forsyth & others, 1971) elicited an increase in blood pressure which was shown to be mediated by the sympathetic nervous system. But a subpressor infusion of angiotensin for up to seven days was without effect on blood pressure of the rats we used. In addition, the infusion of angiotensin in either pressor or subpressor dose was without effect on cardiac catecholamine turnover. This last observation is in contradiction with that of Volicer & Visweswaram (1970) who reported that a subpressor infusion of angiotensin increased cardiac catecholamine turnover in the rat. There is no explanation for this discrepancy. However, a positive control was used in the present study to validate the turnover assay.

In addition to long-term infusion, the method described also allows remote intravenous injection of drugs in unanaesthetized freely-moving rats. When hydralazine was so administered it lowered the blood pressure and concomitantly increased the heart rate. This is consistent with the observations of Ablad (1963) who reported the drug to act primarily on vascular smooth muscle, thus, it lowered blood pressure by decreasing peripheral resistance. It is likely that the concomitant increase in heart rate was a compensatory response mediated, in part, by the baroreceptor reflexes (Brunner & others, 1967). Mecamylamine, a ganglionic blocking agent, also lowered blood pressure, but it had no significant effect on heart rate. It therefore exerted its depressor action by decreasing tonic sympathetic stimulation to the cardiovascular system. Since ganglionic blockade also interrupts the efferent side of the baroreceptor reflex, this accounts for the drug's lack of effect on heart rate.

Clonidine elicited a biphasic blood pressure response. There was a transient increase followed by a prolonged decrease to below preinjection values. This pattern has been observed in a number of species (Boissier, Giudicelli & others, 1968) as well as in man (Onesti, Schwartz & others, 1969). However, Pals (1975), using unanaesthetized rats, found clonidine to be without a depressor effect unless the rats had been sodium depleted. The only apparent difference between Pals' and the present study is that Pals allowed his rats to recover from surgery for 1 h, we allowed 24 to 48 h. Thus, it is possible that surgical stress or the presence of low concentration of anaesthetic interfered with the depressor action of clonidine in Pals' study.

In the presence of hydralazine, the size of pressor response to noradrenaline, angiotensin and tyramine was decreased. With noradrenaline and tyramine, this finding is in agreement with previous observations (Page & McCubbin, 1953). However, the effect of hydralazine on angiotensin-induced vasoconstriction could not be predicted since earlier reports indicate that it varies with species and preparation used. For example, the vasoconstrictor response to angiotensin is not affected by hydralazine in the anaesthetized dog (Page, McCubbin & others, 1957) or in perfused innervated dog kidneys (Page & McCubbin, 1953), but is reduced in the anaesthetized rat and in rabbit isolated hind limb (Meier, Tripod & Studer, 1958). Our data are in agreement with those obtained in anaesthetized rats.

In contrast to hydralazine, mecamylamine inincreased the size of the blood pressure response to all three pressor agents. This action is shared by other ganglionic blocking agents and has been used in bioassays for pressor activity (Sealey, Gerten-Banes & Laragh, 1972). The mechanisms underlying this phenomenon include elimination of the sympathetically and parasympathetically mediated baroreceptor reflexes (Page & McCubbin, 1959, 1963). In addition, there is evidence that mecamylamine sensitizes vascular smooth muscle to the action of vasoconstrictor agents (Robinson & Rama Sastry, 1976). While mecamylamine enhanced the size of response to the three pressor agents, the maximum blood pressure achieved in its presence did not exceed that in its absence. Baseline control blood pressure was 100 mm Hg and was raised on average to 140 mm Hg by the three pressor agents. On the other hand, the baseline blood pressure in the presence of mecamylamine was 62 mm Hg and was raised to 130 mm Hg.

Clonidine had no significant effect on the size of pressor response to noradrenaline or tyramine. In contrast, it increased the pressor response to angiotensin to 173% of control. This agrees with Kobinger & Pichler (1975) who found clonidine to increase the pressor response to angiotensin in the decerebrate rat. However, it appears that the mechanism underlying the interaction between clonidine and angiotensin is specific to these agents since, in the present study, clonidine was without affect on the response to noradrenaline or tyramine.

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