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THE PHARMACOLOGY AND TOXICOLOGY OF SERPAJMALINE

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Serpajmaline is a pale brown powder, readily soluble in water, obtained from the fresh roots of *Rauwolfia serpentina*;[1,2] it contains the alkaloids serpentine, serpentinine and ajmaline, as well as two unidentified substances and some carbohydrate-like material. Serpajmaline contains neither reserpine nor rescinnamine.

Deininger[3] claimed that serpajmaline was a more potent hypotensive drug than reserpine and, further, that it did not have the latter's central sedative effects.

Deininger[4] later investigated the action of serpajmaline on anaesthetised cats and on conscious and anaesthetised dogs and studied its effects on the ileum, both isolated and in situ.

In this paper we record the results of further pharmacological and toxicological experiments on serpajmaline.

Methods

Studies in Conscious Animals

1. *Acute Toxicity*

Mice.—The acute toxicity of serpajmaline was determined by the intravenous, intraperitoneal and oral routes on female fawn mice (GFF strain), and by the oral route in female albino mice (A2G strain). The mice weighed between 16 and 22 g. Serpajmaline solutions were freshly prepared in distilled water immediately before use, and each dose was administered to a group of not less than five mice. Percentage mortalities were assessed seven days after administration, and the LD 50 values were determined by the method of de Beer.[5]

Rats.—The acute toxicity of serpajmaline was determined by the intravenous, intraperitoneal and oral routes on male rats (WAG strain, 100 to 150 g.).

The drug was administered to groups of five rats, and the percentage mortalities were assessed seven days later.

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2. *Subacute Toxicity*

The subacute toxicity of serpajmaline was investigated in albino mice (A2G strain).

Groups of five male and five female mice received daily oral doses of serpajmaline for twelve weeks. The dose was 125 mg./kg., which is approximately one-fifth the oral LD 50 value for this strain. Each mouse was weighed daily; the oxygen consumption rate of each group was measured daily during the third week of treatment only. Two control groups, each of five mice, were included in this test. After twelve weeks' treatment the group oxygen consumption were measured on two consecutive days and the mice were then paired for breeding. They were not dosed with serpajmaline during the breeding period.

3. *Effect on Barbiturate Activity in Mice*

One hundred and fifty male mice (GFF strain, 16 to 26 g.) were injected intraperitoneally with serpajmaline (50 mg./kg. one-fifth LD 50). At intervals ranging from one hour to seven days after receiving serpajmaline, the mice were injected intraperitoneally with hexobarbitone (100 mg./kg.,5 mice per group) or intravenously with barbitone (275 mg./kg., 10 mice per group). The sleep-times of the mice receiving hexobarbitone were measured in a cabinet thermostatically controlled at 37 °C.; the induction periods of the barbitone-injected mice were measured at room temperature.

Control groups, receiving barbiturates only, were included with each experiment to allow for day-to-day variations in barbiturate response.

4. *Analgesic Activity in Mice*

Serpajmaline was tested for analgesic activity in mice by the method of Woolfe and Macdonald.[6] Two groups of ten male mice (GFF strain, 17 to 22 g.) were injected intraperitoneally with serpajmaline (10 or 100 mg./kg.). At intervals after the injection, ranging between 10 minutes and 3 hours, the mice were placed individually on a hot plate maintained at 55 °C. and their reaction times were recorded. The reaction time is defined as the intervals between placing the mouse on the

hot plate and observing signs that indicate the onset of pain, e.g., kicking, paw retraction.

5. Effect on the Blood Pressure of Renal Hypertensive Rats

The rats used in these experiments were made hypertensive by a modification of the renal clamping technique first described by Goldblatt.[7] Young rats of both sexes (40 to 100 g.) were anaesthetised with ether, and a small silver clamp was placed on one renal artery. This reduced but did not completely prevent the flow of blood to the kidney. On recovering from the anaesthetic the rats were maintained in an environment at 28° C. and given free access to a commercial rat cube diet[8] and tap water. Two to three months later a stable chronic renal hypertension was apparent in about 70% of the rats; those that did not become hypertensive (i.e. their blood pressure values were < 140 mm. Hg.) were rejected.

The blood pressures of the hypertensive rats were determined at regular intervals by the tail plethysmographic method of Byrom and Wilson.[9] Blood pressures were assessed under light ether anaesthesia; because of this the determinations were made at intervals of not less than three hours.

Five groups of ten renal hypertensive rats were used in this experiment. One group served as controls, and the other four groups were injected intraperitoneally with serpajmaline on four consecutive days. Group 2 received individual doses of 6.25 mg./kg., and groups 3, 4 and 5, 12.5, 25 50 mg./kg., respectively. Blood pressure recordings were made one hour after each injection and 24 hours and 4 days after the fourth dose.

6. Brain Serotonin-Releasing Activity

Nine adult rabbits were used in this experiment. Three served as controls, three received serpajmaline (10 mg./kg.) and three reserpine (1 mg./kg.). All injections were made into the marginal ear veins. Twenty-four hours later the rabbits were killed and their brains were removed, weighed and assayed spectrofluorometrically for total serotonin, by the method of Bogdanski et al.[10]

7. Effect on Blood Sugar Concentration

Three adult rabbits were injected intravenously with serpajmaline (10 mg./kg.); blood samples were taken before, and 5, 15, 30 and 60 minutes after the injection. Blood sugar levels were estimated by the method of Hagedorn and Jensen.

8. Effect on Clotting Time

Three adult rabbits were injected intravenously with serpajmaline (10 mg./kg.). The rabbits were bled before injection and at intervals thereafter, the samples being collected in waxed stoppered tubes. Clotting times were determined to the nearest half-minute, by inverting the tubes once every 30 seconds.

9. Neuromuscular Blocking Activity in Chicks

Serpajmaline was tested for neuromuscular-blocking activity in conscious chicks by the method of Buttle and Zaimis.[11] All injections were given intravenously.

10. Reserpine-like Action in Kittens

One male and one female kitten (900 to 1000 g.) received single oral doses of serpajmaline (50 mg./kg.). The powder was administered in gelatine capsules. The kittens were observed for 6 hours for evidence of parasympathomimetic activity typical of reserpine.

11. Tranquillising Effect in Monkeys

Two female cynomolgus monkeys (3 to 4 kg.) were given serpajmaline, one animal receiving 5 mg./kg., intravenously and the other 50 mg./kg. orally. Two other monkeys in the same weight range received reserpine (1 mg./kg.), either intravenously or orally.

STUDIES ON ANAESTHETISED ANIMALS

1. Cats

Cats weighing between 2 and 5 kg. were anaesthetised with chloralose (80 mg./kg. intraperitoneal route); blood pressure, respiration and E.C.G. were recorded. The vagus and cervical sympathetic nerves were separated and the responses of the cardiovascular system to peripheral vagal stimulation and of the nictitating membrane to peripheral sympathetic stimulation were recorded. A cannula was inserted into the saphenous vein and injections of saline, adrenaline, noradrenaline, acetylcholine and histamine were administered intravenously.

In two experiments serpajmaline was administered into the lateral cerebral ventricle via a Feldberg cannula,[12] and in another experiment the effects of serpajmaline on the motility of the small intestine in situ were recorded by the method of Straub.

Serpajmaline was administered by intravenous injection, intravenous infusion, intraventricular injection, intraduodenal injection and via an oesophageal catheter directly into the stomach. In several experiments serpajmaline was also injected through a catheter passing down the external jugular vein with its tip terminating at the point where the great veins enter the right auricle.

2. Dogs

Mongrel bitches weighing between 8 and 12 kg. were anaesthetised intravenously or intraperitoneally with chloralose (100 mg./kg.). When necessary anaesthesia was maintained with small doses of sodium pentobarbitone.

The blood pressure, respiration and E.C.G. were recorded, and the drugs were injected either through a cannula in the femoral vein or directly into the duodenum. The responses of the cardiovascular system to peripheral vagal stimulation and of the nictitating membrane to pre-ganglionic stimulation were recorded.

3. Monkeys

Serpajmaline and reserpine were compared in six female cynomolgus monkeys weighing between 3 and 4 kg. The animals were anaesthetised with sodium pentobarbitone (30 mg./kg. intravenously), and the blood pressure, respiration and E.C.G. were recorded. All injections were given through a cannular inserted in the femoral vein.

4. Rabbits

Rabbits were anaesthetised with urethane (1 g. /kg.) injected intraperitoneally, and the blood pressure and respiration were recorded. All drugs were injected into the femoral vein.

STUDIES ON ISOLATED TISSUES

In vitro effects of serpajmaline were studied on guinea-pig ileum, rabbit duodenum, non-oestrus rat uterus, chick semispinalis cervicis muscle, perfused rabbit heart and beating rabbit auricles.

1. Guinea-pig Ileum

The ileum was suspended in a 20 ml. bath of oxygenated Tyrode solution at 31 °C. Longitudinal contractions were recorded with a frontal writing lever on a smoked drum.

2. Rabbit Duodenum

The duodenum was removed from a freshly killed rabbit and suspended in oxygenated Tyrode solution at 35 °C.

3. Rat Uterus

One horn of the uterus of a non-oestrus rat was suspended in a bath of de Jalon solution at a temperature of 31 °C. The solution was oxygenated with 95% oxygen and 5% carbon dioxide.

4. Chick Semispinalis Muscle

One of the semispinalis cervicis muscles of the chick was removed under ether anaesthesia and suspended in a bath of oxygenated Tyrode solution at 40.5 °C. Longitudinal contracture of the muscle was recorded with a frontal writing lever.

5. Perfused Beating Rabbit Heart

Rabbit hearts were perfused by a modification of the Langendorff technique. The hearts were perfused with McEwen solution[13] at 37°C. and at a perfusion pressure of 40 cms. of water. The perfusion fluid when fully saturated with a mixture of 95% oxygen and 5% carbon dioxide had a pH of 7.4. Coronary flow was recorded with an inflow recorder, and the amplitude was recorded on a smoked drum. Drugs were added to the perfusion fluid via a polythene cannula opening near the coronary arteries so that dilution was reduced to a minimum.

6. Perfused Fibrillating Rabbit Heart

Fibrillation was induced in perfused beating rabbit hearts by electrically stimulating the ventricle and reducing the concentration of potassium in the McEwen solution to three-quarters of the normal amount. After fibrillating for 15 minutes a perfused heart never spontaneously reverted to normal rhythm; further, an injection of sodium chloride, potassium chloride or distilled water from pH 5 to pH 8 did not affect the fibrillation.

7. Rabbit Auricles

The effect of drugs on the maximum drive rate of the rabbit auricles was determined by the method of Dawes[14] as modified by Alles and Ellis.[15]

The heart was removed from a freshly killed rabbit and the auricles were dissected free from other heart muscle and suspended in a bath of oxygenated (95% oxygen and 5% carbon dioxide) Locke solution at a temperature of 29°C. The auricles were stimulated electrically with square wave pulses of 3 m.secs width and an intensity of

Table 1.—The Acute Toxicities of Serpajmaline in Mice and Rats.

Species	Strain	Sex	LD 50 value — mg./kg.		
			Intravenous	Intraperitoneal	Oral
Mouse	GFF	Female	29	240	760
Mouse	A2G	Female	Not tested	Not tested	660
Rat	WAG	Male	52	230	> 1100

Table 2.—The Effects of Serpajmaline on Weight Increase in Mice (A2G).

Group	Number in group	Group mean body weight (g.)												Per cent increase in body weight
		Weeks of treatment												
		Initial	1	2	3	4	5	6	7	8	9	10	11	12	
Test—Males ..	4*	8.5	13.1	17.4	20.1	20.4	22.0	23.3	24.5	24.3	26.5	27.8	26.6	27.3	222†
Control—Males ..	5	8.6	14.2	18.5	21.7	23.7	24.4	25.8	26.2	27.0	27.9	28.9	29.2	30.4	253
Test—Females ..	5	9.3	12.9	15.7	17.4	18.3	18.4	19.1	20.3	20.6	21.6	22.5	22.1	22.7	144†
Control—Females	5	9.1	13.8	16.9	18.9	19.6	19.4	20.9	21.2	21.8	22.5	23.4	23.3	24.0	164

* One animal died at the beginning of the experiment through faulty administration technique.

† The depressions in growth rate are significant $0.1 > P > 0.05$).

Table 3.—The Group Oxygen Consumption Rates of Mice after Daily Oral Dosing with Serpajmaline.

Group	Number in group	Group oxygen consumption rate litres/kg./hour						
		Days of treatment						
		15	16	17	20	21	85	86
Test—Males	4*	4.02	3·71	3·71	3.13	3.97	3.88	3.59
Control—Males	5	4.83	4.81	4.02	3.51	3.87	3.97	3.86
Test—Females	5	4.77	4.36	3.52	3.00	4.11	4.11	3.72
Control—Females	5	4.50	3.67	3–34	3.55	3.73	3.29	3.13

* See Table 2.

4 to 6 volts., (twice the threshold value determined at a stimulation rate of 180 per minute).

The normal heart rate and the maximum drive rate were determined immediately before and immediately after the auricles had been in contact with the drug for 20 minutes.

Results

STUDIES IN CONSCIOUS ANIMALS

1. *Acute Toxicity*

The results are given in Table 1.

In order to know whether the acute toxicity of serpajmaline changed on standing, the acute intravenous toxicity of a 0.3% w/v solution of serpajmaline was determined on female mice (GFF strain, 16 to 20 g.) immediately after preparation and after standing at room temperature (ca. 20°C.) for 3, 6, 24 and 168 hours.

There were no changes in the intravenous LD 50 during the seven days of the experiment, the values ranging from 27 to 29 mg./kg.

2. *Subacute Toxicity*

The group mean bodyweights obtained during the twelve weeks of treatment are given in Table 2 and the group oxygen consumption rates during the third week and at the end of treatment are shown in Table 3.

The group oxygen consumption rates of mice during and after treatment with serpajmaline do not differ from those of the untreated controls. Twelve weeks treatment with the serpajmaline slightly depressed ($0.1 > p > 0.05$) the growth rate in both the males and the females, but in no way affected fertility in males or females (Table 4).

3. *Effect on Barbiturate Activity in Mice*

The results together with those previously obtained in a similar experiment with reserpine are given in Table 5. It will be seen that serpajmaline had no effects on the activities of the two barbiturates in mice.

4. *Analgesic Activity in Mice*

The group mean reaction times after serp-

TABLE 5.—THE EFFECTS OF INTRAPERITONEAL INJECTIONS OF SERPAJMALINE OR RESERPINE ON BARBITURATE ACTIVITY IN MICE.

Time after injection	Serpajmaline (50 mg./kg.)				Reserpine (2 mg./kg.)			
	Hexobarbitone sleep time Mean ± S. D.* (mins.)		Barbitone induction-time Mean ± S. D.† (mins.)		Hexobarbitone sleep time Mean ± S. D.* (mins.)		Barbitone induction-time Mean ± S. D.† (mins.)	
	Test	Control	Test	Control	Test	Control	Test	Control
1 hour	21 ± 4	23 ± 3	16.9 ± 1.1	19.2 ± 2.6	33.4 ± 10.9	26.0 ± 5.6	8.0 ± 1.3	19.5 ± 2.9
2 hours	Not tested	..	Not tested	..	69.8 ± 28.8	26.0 ± 5.6	4.9 ± 0.7	19.5 ± 2.9
3 „	26 ± 4	23 ± 3	17.1 ± 2.3	19.2 ± 2.6	92.4 ± 21.0	23.2 ± 1.9	3.2 ± 0.9	19.5 ± 2.9
6 „	21 ± 2	23 ± 3	17.3 ± 1.3	19.2 ± 2.6	75.8 ± 11.7	22.3 ± 1.9	3.8 ± 1.3	20.0 ± 2.9
1 day	24 ± 4	25 ± 5	20.2 ± 1.5	19.2 ± 2.6	30.4 ± 6.6	24.8 ± 5.2	12.9 ± 5.0	19.2 ± 1.7
2 days	24 ± 5	25 ± 5	23.0 ± 2.4	24.4 ± 2.1	15.0 ± 3.4	16.6 ± 4.0	20.3 ± 2.1	18.3 ± 2.1
3 „	16 ± 3	25 ± 5	21.3 ± 1.4	22.5 ± 1.5	12.3 ± 3.3	22.3 ± 1.8	34.5 ± 3.6	17.7 ± 1.5
4 „	28 ± 5	25 ± 3	22.8 ± 2.6	20.5 ± 2.5	11.4 ± 2.7	24.8 ± 5.2	31.3 ± 8.4	18.1 ± 3.8
5 „	24 ± 2	25 ± 3	22.4 ± 1.3	21.7 ± 2.2	12.0 ± 3.2	16.6 ± 4.0	22.6 ± 2.6	18.3 ± 2.8
6 „	19 ± 5	25 ± 3	19.7 ± 1.2	20.5 ± 2.5	17.6 ± 6.1	23.0 ± 2.9	20.2 ± 2.3	19.0 ± 1.4
7 „	Not tested	..	21.3 ± 1.4	21.7 ± 2.2	22.2 ± 2.5	27.0 ± 7.0	19.0 ± 3.5	18.0 ± 3.8

* 5 mice per group. † 10 mice per group.

ajmaline together with the control times are given in Table 6.

It will be seen that serpajmaline had no analgesic action even at a dose level equivalent to almost half the LD 50 value. Results obtained in a similar experiment with pethidine hydrochloride are included for comparison.

5. Effect of Repeated Doses of Serpajmaline on Blood Pressure in Renal Hypertensive Rats

The results are illustrated in Fig. 1. There were no changes in response over the four days when doses between 6.25 and 25 mg./kg. were used. There may have been slight cumulation, however, at a dose level of 50 mg./kg.

6. Brain Serotonin-Releasing Activity

Shore, Pletscher, Tomich, Carlsson, Kuntzman and Brodie[16] have shown that reserpine injected intravenously into rabbits rapidly reduces the level of serotonin in the brain. The low level of brain serotonin persists for about 40 hours and is finally restored to its normal value after 5 or 6 days. In the rauwolfia group of alkaloids only the tranqu-

TABLE 4.—RESULTS OF BREEDING EXPERIMENTS.

Group	Number of pairs	Number of pairs having litters	Number of young born	Number of young reared
Test	5*	5*	27	21†
Control	5	5	26	21†

* One of the males served two females as only 4 test males were available.
† One whole litter killed at birth.

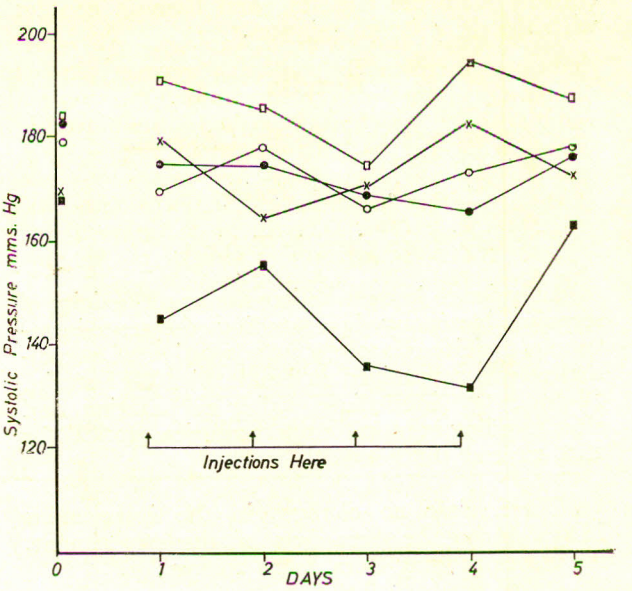

Fig. 1.—The effect of serpajmaline on systolic blood pressure in renal hypertensive rats. Intraperitoneal injections were given on four successive days and the blood pressure recorded one hour after each injection.

● Control animals.
□ 6.25 mg./kg. serpajmaline.
× 12.5 „ „
○ 25 „ „
■ 50 „ „

TABLE 6.—TESTS FOR ANALGESIC ACTIVITY USING SERPAJMALINE AND PETHIDINE IN MICE (ALL INJECTIONS GIVEN INTRAPERITONEALLY).

Drug	Dose mg./kg.	Mean reaction time ± S. D * (seconds) Minutes after injecting drug					
		Initial	10	30	60	120	180
Serpajmaline	10	6 ± 2	7 ± 3	8 ± 3	7 ± 2	7 ± 2	7 ± 2
Serpajmaline	100	6 ± 2	11 ± 3	9 ± 3	10 ± 3	11 ± 3	10 ± 2
Pethidine	50	8 ± 2	43 ±17	28 ±15	17 ±10	Not tested	Not tested

* 10 mice per group.

illising members are able to release brain serotonin, and Brodie and his colleagues have suggested that these two activities may be associated. Hence it seemed of interest to ascertain the effect of serpajmaline on brain serotonin.

The results are given in Table 7. All results have been corrected for percentage recovery, known amounts of serotonin being added to normal rabbit brain, which was assayed simultaneously. These recoveries varied from 48 to 70%.

TABLE 7.—THE EFFECTS OF SERPAJMALINE AND RESERPINE ONE DAY AFTER ADMINISTRATION ON BRAIN-SEROTONIN LEVELS IN RABBITS (BOTH DRUGS INJECTED INTRAVENOUSLY).

Brain serotonin concentration (μg./g.)		
Controls	Serpajmaline (10 mg./kg.)	Reserpine (1 mg./kg.)
0.53	0.70	0.09
0.52	0.56	0.11
0.58	0.60	0.10
Average: 0.53	0.62	0.10

The normal serotonin concentration in rabbit brain is 0.52 to 0.58 μg./g. fresh tissue. Twenty-four hours after reserpine (1 mg./kg.) the level fell to 0.09 - 0.11 μg./g., but serpajmaline (10 mg./kg.) had no effect.

7. Effect on Blood Sugar Concentration

An intravenous injection of reserpine (5 mg./kg.) into the rabbit increased the blood sugar level within one hour of administration.[17] Serpajmaline had no effect on blood sugar when injected intravenously at 10 mg./kg.

8. Effect on Blood Clotting Time

Serpajmaline did not influence clotting time in the rabbit.

9. Neuromuscular-Blocking Activity in Chicks.

Intravenous injections into the chick of neuromuscular-blocking drugs produce characteristic patterns of muscular paralysis, which differ with the type of drug employed.[11] Thus "depolarising blockers" (e.g. decamethonium) produce a period of spasticity in the hind limbs and retraction of the head, whereas "competitive blockers" (e.g. *d*-tubocurarine) produce flaccid paralysis only. Both syndromes become apparent immediately after injection. Blocking agents of the third type, the anticholinesterases, produce a "depolarising block" resulting from the accumulation of of acetylcholine. The pattern of spasticity is similar to that observed with decamethonium, but there is a delay before onset, its length depending on the dose administered.

Intravenous injections of serpajmaline in doses from 2 to 4 mg./kg. were without effect. Higher doses (5 to 10 mg./kg.) produced slight ataxia, which was enhanced by covering the chick's eyes. Serpajmaline administered as a single intravenous dose of 15 mg./kg. was lethal to chicks.

Doses of decamethonium, *d*-tubocurarine and neostigmine required to produce characteristic neuromuscular block in the chick are 50, 500 and 1000 μg./kg. respectively.

Serpajmaline does not show neuromuscular-blocking or anticholinesterase activity in the chick.

10. Reserpine-like Activity in Kittens

Serpajmaline (50 mg./kg. orally) had no apparent effect on conscious kittens. There were no signs of gastric discomfort, diarrhoea, anorexia, sedation or relaxation of the nictitating membrane. Such responses are readily obtained with reserpine in doses as low as 0.5 mg./kg.

11. Tranquillising Effect in Monkeys

Within one hour of receiving reserpine, both monkeys became docile, approached the front of the cage and were obviously sedated. This condition was still apparent three hours later, but by 24 hours both had resumed their characteristically hostile attitude. Diarrhoea occurred in the monkey receiving reserpine by the intravenous route, but not in the one treated orally.

The two monkeys treated with serpajmaline remained hostile throughout the experiment. There were no signs of sedation or obvious discomfort in either animal. The serpajmaline did not appear to cause pain when injected into the femoral vein.

STUDIES IN ANAESTHETISED ANIMALS

1. Cats

Intravenous Injection.—Small doses of serpajmaline (0.25 to 2.0 mg./kg.) had no effect on

blood pressure, respiration, E.C.G. or the cardiovascular responses to adrenaline, noradrenaline, acetylcholine or histamine. The response of the nictitating membrane to pre-ganglionic stimulation remained unimpaired, but the fall in blood pressure and the bradycardia produced by peripheral vagal stimulation were completely abolished by serpajmaline in doses greater than 1 mg./kg. However, the apnoea resulting from central vagal stimulation remained unaffected. The vagal-blocking action of serpajmaline was reversible when doses of 1 or 2 mg./kg., were given, the response returning to normal within two hours.

The smallest dose of serpajmaline (0.25 mg./kg.) increased the tone and motility of the gut for about two minutes, and these effects increased in severity but not in duration with increasing dose.

With larger doses of serpajmaline (5 to 10 mg./kg.) a fall in blood pressure was produced; its degree and duration depended on the initial blood pressure level. In general, a fall of between 40 and 60 mms. Hg was obtained, the pressure returning to normal within 20 minutes. The fall in blood pressure was associated with depression of respiration, slight bradycardia, increase in the P-R interval of the E.C.G., reduction in the pressor response to noradrenaline and reversal of the pressor response to adrenaline. In addition, the reflex bradycardia normally obtained at the height of the noradrenaline response was abolished. The response of the nictitating membrane to adrenaline was reduced, and that to supra-maximal pre-ganglionic stimulation was sometimes reduced and sometimes unaffected (Fig.2). Peripheral vagal stimulation was blocked, but the apnoea resulting from central vagal stimulation remained unaltered. The blocking action of these doses on the effects of peripheral vagal stimulation was irreversible during the experimental period of four to five hours. The vascular responses to acetylcholine and histamine remained unchanged by serpajmaline in doses of 10 mg./kg.

Doses greater than 10 mg./kg. produced acute respiratory depression and death, when the heart remained in diastole.

Intravenous Infusion.—Most of the effects described above could be obtained by intravenously infusing serpajmaline at a rate of 0.5 mg./kg./minute. A complete block of the responses to peripheral vagal stimulation was obtained long before any effects on blood pressure became apparent (Fig.3). At this rate of infusion the responses of the nictitating membrane to preganglionic stimulation and to injected adrenaline were reduced by about 50 per cent. The blood pressure fell by about 40 mm.

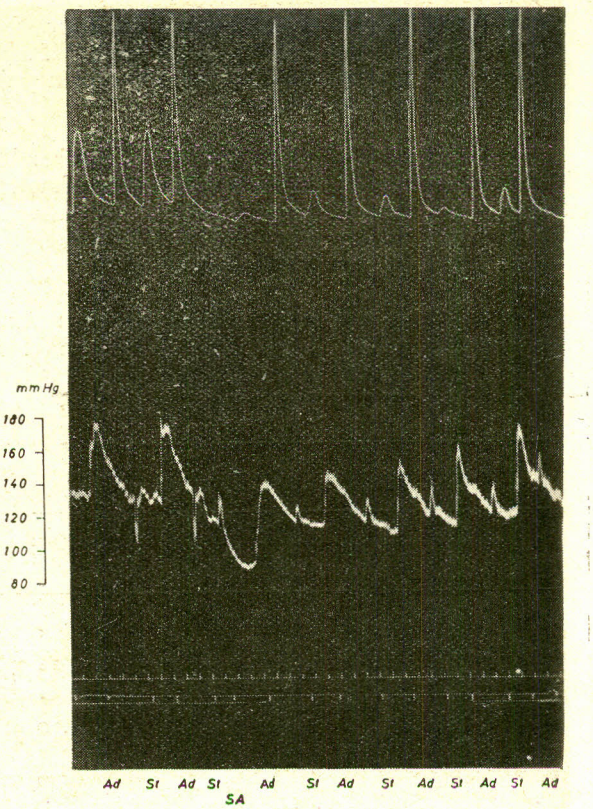

Fig. 2.—The effect of serpajmaline on the blood pressure and nictitating membrane of the anaesthetised cat (2.3 kg., chloralose 80 mg./kg. intraperitoneally). Intravenous injections of 10 μg. adrenaline at "Ad" and supramaximal stimulation of the vagosympathicus at "St". Serpajmaline, 8 mg./kg., injected intravenously at "SA".

Hg, bradycardia was observed, and the pressor response to noradrenaline was reduced and that to adrenaline was reversed. The depressor response to injected acetylcholine was not altered.

On stopping the infusion, the blood pressure rapidly returned to normal. A second infusion conducted at the same rate in the same animal produced a second fall in blood pressure, which was still obtained after cutting both vagi.

Respiration was not depressed with this rate of infusion, but with more rapid infusion rates some respiratory depression was observed. In a cat maintained under artificial respiration a total dose of serpajmaline of 100 mg./kg. infused at a rate of 1 mg./kg./minute was not fatal.

Injection Close to the Heart.—The fall in blood pressure and the bradycardia produced by peripheral vagal stimulation were completely blocked by

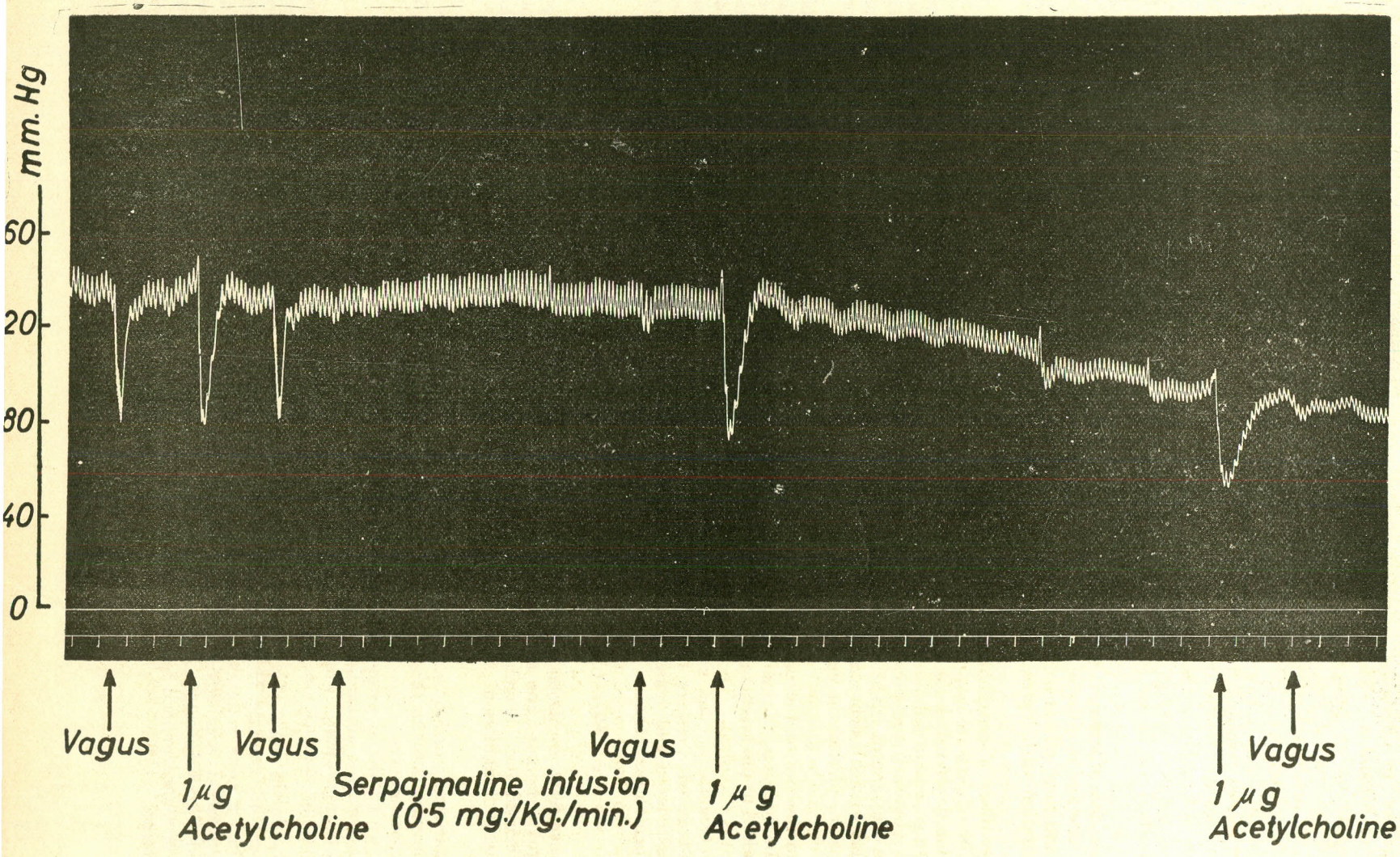

Fig. 3.—Effect of intravenous infusion of serpajmaline on the blood pressure of the anaesthetised cat (3.0 kg., chloralose 80 mg./kg. intraperitoneally). Vagus stimulated with supramaximal stimuli at "V" and acetylcholine 1 μg. injected intravenously at "Ach". Serpajmaline infusion started at "SA" (0.5 mg./kg./minute).

injecting 50 μg. of serpajmaline close to the point where the great veins enter the right auricle.

Injection into the Lateral Cerebral Ventricle.—Doses from 50 to 400 μg. administered directly into the lateral cerebral ventricle had no pharmacological actions in anaesthetised cats. Under these conditions reserpine (25 to 50 μg.) depressed both respiration and blood pressure.

Injection Directly into the Duodenum or Administration via Oesophageal Catheter into the Stomach.—Administration of serpajmaline into either the stomach or the duodenum of anaesthetised cats produce a fall in blood pressure after a latent period of 15 to 30 minutes. The fall in pressure, although small (20 to 40 mms. Hg) was attributed to the drug and not to deterioration in the animal's condition, the fall being associated with a blockage of peripheral vagal stimulation and a reduction in the pressor responses to adrenaline and noradrenaline. The drug did not depress respiration or alter the responses of the nictitating membrane to adrenaline or to supra-maximal pre-ganglionic stimulation. In one or two experiments the blood pressure returned to the pre-injection level, but usually it was still depressed at the end of the experiment.

Serpajmaline was not very active by the oral or intraduodenal routes, a dose 50 to 100 mg./kg. being required to produce pharmacological effects.

In two cats 100 mg./kg. administered into the stomach produced a rise in blood pressure. In the remaining six cats so treated this dose of serpajmaline was hypotensive.

2. *Dogs*

Intravenous Injection.—Doses of serpajmaline less than 5 mg./kg. did not lower the blood pressure of the anaesthetised dog. A complete block of peripheral vagal stimulation was obtained with 2 mg./kg.

Doses between 5 and 10 mg./kg. produced falls in blood pressure between 50 and 70 mms. Hg and lasting for 30 minutes. The fall in pressure was associated with reduced pressor responses to noradrenaline, reversed adrenaline responses and a depression of respiration. The fall in blood pressure produced by acetylcholine was unchanged.

Serpajmaline in doses of 20 mg./kg. produced a long lasting fall (100 mms.Hg) in blood pressure. The respiration was completely depressed, but normal respiration was again apparent after a short period of artificial respiration (2 to 5 minutes). The blood pressure remained depressed throughout the experiment.

Intraduodenal Injection—Serpajmaline in doses less than 10 mg./kg. had no effect when administered intraduodenally into anaesthetised dogs. Doses of 10 or 20 mg./kg. produced falls in blood pressure (about 30 mms. Hg) after a latent period of 15 minutes. The depressor effect lasted from one to two hours.

3. *Monkeys*

Doses of serpajmaline less than 2 mg./kg. were without effect, but 2 or 4 mg./kg. produced a small fall in blood pressure and bradycardia, and blocked the effects of peripheral vagal stimulation. Higher doses (5 to 10 mg./kg.) produced a fall in pressure of approximately 50 mms. Hg, which lasted for 30 minutes. The pressor response to noradrenaline was reduced; that to adrenaline was blocked but not reversed.

Fifty mg./kg. given in divided doses (5, 10, 15 and 20 mg./kg.) at five minute intervals produced a sustained fall in blood pressure lasting for more than two hours, partial recovery occurring during the third hour. In this monkey the fall in pressure was associated with slight respiratory depression.

In similar experiments a single dose of reserpine of 5 mg./kg. produced a sustained fall in blood pressure (70 to 80 mms. Hg) lasting throughout the experimental period of 3 to 4 hours. Respiration in all three monkeys injected with reserpine was depressed, but no reductions in the pressor responses to adrenaline and noradrenaline were observed, and the effects of peripheral vagal stimulation were not blocked.

4. *Rabbits*

Serpajmaline has a smaller depressor activity in rabbits than in other species. Five mg./kg. produced only a transient fall in blood pressure; a dose of 10 mg./kg. was lethal.

Twenty mg./kg. administered in four doses of 5 mg./kg. at five minute intervals produced a fall in blood pressure of 50 mms. Hg.

STUDIES ON ISOLATED TISSUES

Guinea-pig Ileum.—At concentrations less than 10^{-5} serpajmaline had no effect on guinea-pig ileum, neither did it antagonise the actions of barium chloride or acetylcholine. At higher concentrations (1.3 to 1.6×10^{-5}) it increased the spontaneous activity of the ileum, but depressed contractions induced by barium chloride or acetylcholine. However, increased sensitivities to both barium chloride and acetylcholine were apparent after

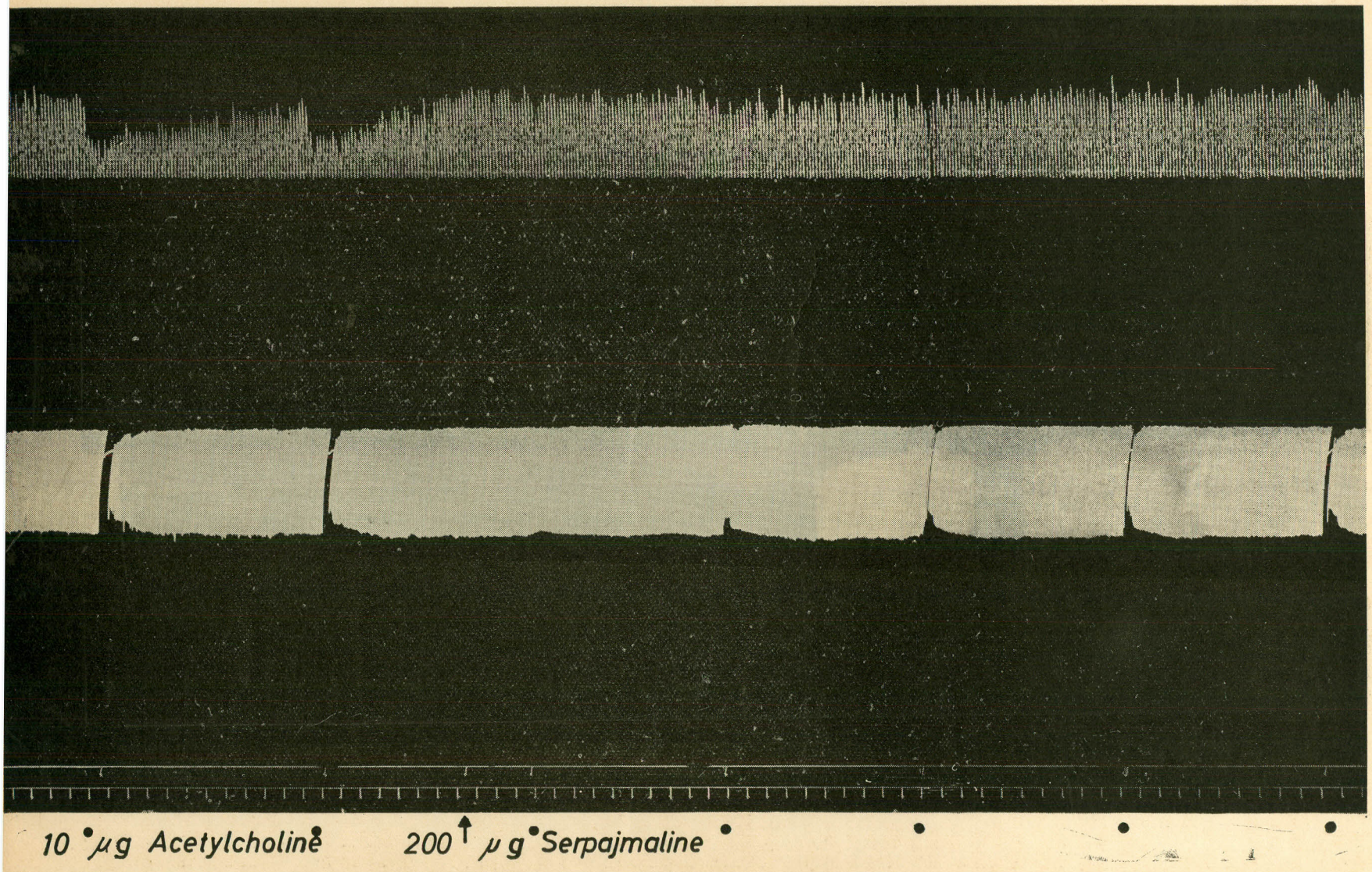

Fig. 4 :—Effect of serpajmaline on the response of the isolated perfused rabbit heart to acetylcholine. Upper tracing: coronory inflow. A decrease in coronory inflow is recorded as a decreased height of tracing. Lower tracing: Amplitude of beat. At 10 µg. of acetylcholine injected into perfusion fluid. At SA 200 µg. serpajmaline injected into perfusion fluid.

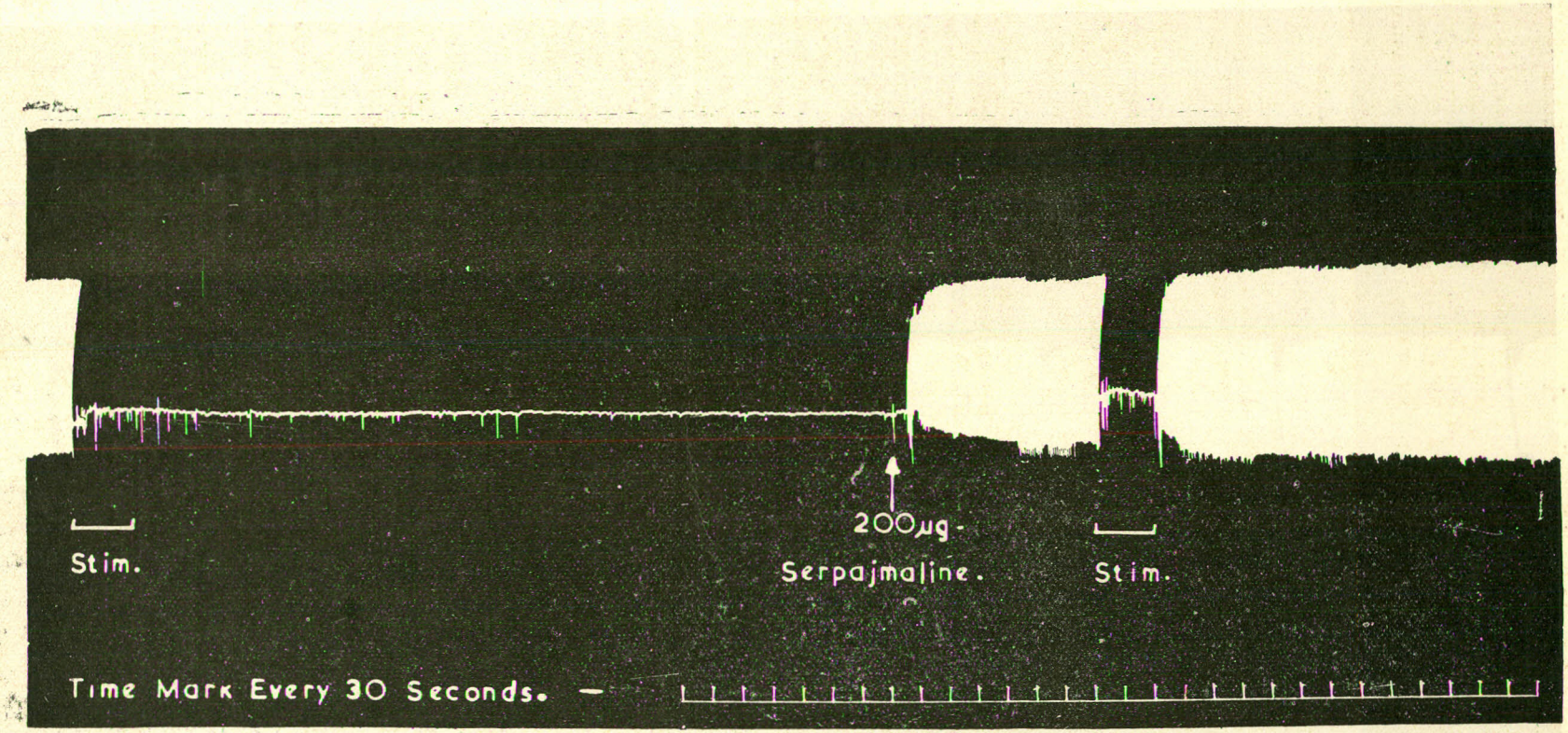

Fig. 5.—Effect of serpajmaline on the isolated perfused fibrillating rabbit heart. At the vertical arrow 200 µg. serpajmaline injected into the perfusion fluid. At "Stim" electrical stimulation applied to the ventricle.

washing the ileum several times with fresh Tyrode solution.

Rabbit Duodenum

Serpajmaline at concentrations of 1.5×10^{-5} did not affect normal peristalsis of rabbit duodenum.

Non-oestrus Rat Uterus

Concentrations of serpajmaline less than 1.5×10^{-5} had no effect on the non-oestrus rat uterus. Higher concentrations (2.5 to 5.0×10^{-5}) immediately produced alternate rhythmic contractions and relaxations of the uterus, which were maintained as long as the serpajmaline remained in contact with it. After washing out the serpajmaline marked increases in the sensitivity of the uterus to the stimulant actions of both carbachol and posterior pituitary extract were observed.

Chick Semispinalis Cervicis Muscle

Serpajmaline had no direct depolarising action on the isolated semispinalis cervicis muscle of the chick. At concentrations greater than 4×10^{-5} it antagonised the depolarising action of decamethonium: in this respect it was 500 times less active than *d*-tubocurarine.

Perfused Beating Rabbit Heart

Doses of serpajmaline less than 200 μg. had no effect on the isolated beating rabbit heart; 200 to 400 μg. slightly decreased the amplitude and reduced the coronary flow for about 3 minutes. The positive inotropic and chronotropic effects of adrenaline were unchanged, but the bradycardia normally produced by acetylcholine was completely abolished and did not return to normal for at least one hour (Fig.4).

Higher doses of serpajmaline (500 to 1000 μg.) produced bradycardia and marked decreases in amplitude and coronary flow.

Perfused Fibrillating Rabbit Heart

Serpajmaline injected into hearts in which fibrillation had been maintained for 15 minutes restored the beat to normal within one or two minutes (Fig.5). The dose required to produce this effect ranged between 100 μg. and 1000 μg., the mean value for 29 hearts being 350 μg.

A qualitatively similar result was obtained with quinidine (mean value 425 μg., range 200 to 800 μg. in 13 hearts).

Rabbit Auricles

Serpajmaline reduces both the normal rate of auricular beat and the maximum rate at which the auricle can respond to electrical stimulation (the effective refractory period). Qualitatively similar results were obtained with quinidine.

Discussion

The pharmacology of serpajmaline is complex, since it is a mixture of carbohydrate with at least five substances, of which three have been identified with known alkaloids.

These three alkaloids, serpentine, serpentinine and ajmaline, have been studied extensively by many workers, and there are wide differences of opinion about their sites and modes of action.

However it is generally agreed that all three reduce blood pressure in the anaesthetised cat and dog, that the fall in pressure is not affected by bilateral vagotomy and that none of the three compounds produces a fall in blood pressure when administered orally to the conscious dog. In addition, all three alkaloids stimulate intestinal musculature and depress respiration, and when injected in high doses into mice they produce convulsions. None of these substances is adrenolytic in the cat, but it is said that serpentinine possesses antiadrenaline activity and abolishes the effects of peripheral vagal stimulation in the anaesthetised dog.

Serpentine possesses slight ganglion blocking activity in the cat, the other two alkaloids being inactive in this respect. Ajmaline is reported to produce a slight rise in the fibrillation threshold in cats, rabbits and frogs without affecting the refractory period or conduction time (for references see 18).

More recently Arora and Madan have investigated the anti-arrhythmic activity of both ajmaline and serpentine and have found both alkaloids more active in this respect than quinidine.[19]

Thus it may be assumed that, though certain pharmacological properties of serpajmaline may be due to its three known constituents, their combined activity cannot account for all its actions.

Serpajmaline, injected intravenously into anaesthetised animals, reduced blood pressure in all the species examined; the dog and the monkey being the most, and the rabbit the least, sensitive of the animals tested.

The blood pressure fall obtained with serpajmaline in the anaesthetised animal is of short duration and is associated with

1. bradycardia
2. block of cardiac responses to peripheral vagal stimulation
3. block and sometimes reversal of the pressor response to adrenaline
4. reduction in the pressor response to noradrenaline
5. reduction in the response of the nictitating membrane to adrenaline
6. variable effect on the response of the nictitating membrane to supra-maximal pre-ganglionic stimulation
7. depression of respiration
8. stimulation of intestinal musculature.

Serpajmaline does not affect the vascular responses to acetylcholine or histamine, neither does it influence the apnoea produced by central vagal stimulation. No depression of blood pressure or respiration is obtained when serpajmaline is injected directly into the lateral cerebral ventricle.

This evidence, and the results of the experiments conducted on conscious animals, suggest that serpajmaline does not depress the central nervous system and has little or no action at the ganglia of the autonomic nervous system. Presumably the fall in pressure produced in anaesthetised animals is more peripheral in origin.

However the fall in blood pressure cannot always be correlated with the anti-adrenaline activity, and it is possible that in some animals serpajmaline produces direct vasodilation.

The cardiac actions of serpajmaline in anaesthetised animals seem to us particularly interesting. Serpajmaline produces bradycardia and yet simultaneously blocks the fall in blood pressure and bradycardia produced by stimulating the peripheral end of the vagus. The bradycardia cannot therefore arise from stimulation of the parasympathetic system or be due to increased sensitivity of the sino-auricular node to normal parasympathetic impulses. It is unlikely that the bradycardia results from decreased sympathetic activity alone, since such a mode of action requires the existence of an intact parasympathetic system.

The results of the experiments on anaesthetised animals and on the isolated perfused rabbit heart preparation indicate that serpajmaline blocks the cardiac actions of acetylcholine without affecting its vascular actions; in this respect it has many similarities with quinidine. Thus many of the actions of serpajmaline in anaesthetised animals may also be obtained with quinidine itself.

Intravenous injections of quinidine sulphate (5 to 10 mg./kg.) in the cat produce a slight short-lasting fall in blood pressure, bradycardia, respiratory depression, blocking of the cardiac actions of peripheral vagal stimulation and reduction in the pressor responses to both adrenaline and noradrenaline, the vascular response to acetylcholine remaining unaltered.

The results obtained with serpajmaline on fibrillating rabbit hearts and on electrically driven auricles show that serpajmaline is more active than quinidine.

Conclusions

1. Serpajmaline is not toxic when administered by the oral route to mice and rats, the LD 50 values being 750 and >1100 mg./kg. respectively.

2. The oxygen consumption rate of mice receiving a daily oral dose of serpajmaline (125 mg./kg.) is not affected after twelve weeks' treatment.

3. The growth rate of mice is slightly depressed ($0.1 > p > 0.05$) after twelve weeks of daily oral dosing with serpajmaline (125 mg./kg.).

4. Serpajmaline is much less active in the renal hypertensive rat, and in anaesthetised animals generally, than it is in the conscious dog.

5. In the anaesthetised animal serpajmaline reduces blood pressure in several ways, of which the most important is probably its anti-adrenaline activity. In some animals a direct vaso-dilator action may occur. Serpajmaline does not possess ganglion-blocking, atropine-like or anti-histamine activity.

6. In conscious mice, rats, rabbits, cats, monkeys, and chicks, serpajmaline has no central sedative, analgesic, neuromuscular-blocking, or anti-cholinesterase activities. It does not affect the activity of barbiturates in mice or the level of serotonin in the brain, the blood sugar concentration or the clotting time in the rabbit.

7. Serpajmaline possesses many of the actions of quinidine, the principal one being a potent antifibrillatory action on the isolated perfused mammalian heart.

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