PHARMACOLOGICAL STUDIES IN THE APOCYNACEOUS GENUS ASPIDOSPERMA MART. & ZUCC., *ASPIDOSPERMA ULEI* MGF.

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THE pharmacological properties of the alkaloids of certain Aspidosperma species have already been discussed.[1,2,3]. This paper deals with the alkaloids of *Aspidosperma ulei* Mgf.

Materials. The dried bark was reduced to a coarse powder and the total alkaloids extracted by classical methods and purified. A 1 per cent. aqueous solution of the recrystallised alkaloidal hydrochlorides was used. Sodium chloride was added to this solution to give a concentration of 0·9 per cent. for mammalian and 0·6 per cent. for frog tissues.

Methods and Results. When applied to the muscle of the frog sciatic nerve-gastrocnemius muscle preparation there was a contractural response which was reversible on washing. There was a progressive decline in the response of the muscle to indirect stimulation, and depression of response when the drug was applied to the sciatic nerve. Inhibition in both cases progressed to completion and was irreversible although the muscle responded to direct stimulation. 0·5 to 1·0 mg. of the alkaloidal hydrochlorides did not antagonise the spasmogenic actions of 10 μg. of acetylcholine chloride on the frog rectus abdominis. Following addition of 0·5 to 1·0 mg. of the alkaloidal hydrochlorides to the bath, a slow contracture developed which was not antagonised by 10 μg. of atropine sulphate, or by 100 μg. of hexamethonium bromide or by 100 μg. of *d*-tubocurarine chloride. The action of the drug was not potentiated on the eserinised preparation.

0·1 mg. of alkaloidal hydrochlorides caused depression of smooth muscle tone with inhibition of peristalsis in rat and guinea-pig ileum and rabbit duodenum. The tone and normal rhythmic movements of rat, guinea-pig and rabbit uterus were inhibited by 1·0 mg. The spasmogenic actions of 1 μg. of acetylcholine chloride on rat and guinea-pig ileum, of 10 μg. on rat and guinea-pig uterus, and of 2 μg. on rabbit duodenum were inhibited by 0·1 mg. of the drug. Inhibition of the spasmogenic actions of 1 μg. of histamine acid phosphate on guinea-pig ileum and uterus by 0·1 mg. was shown. A similar effect was shown for 2 mg. barium chloride at a dose level of 2 mg. of alkaloidal hydrochlorides, using rat and guinea-pig uterus, and rat, rabbit and guinea-pig ileum. On the rabbit uterus, antagonism to the spamogenic action of 10 μg. of adrenaline hydrochloride was shown. In all cases a 50-ml. bath was used containing for rat tissues, oxygenated De Jalon's solution[4], and for others,

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oxygenated Tyrode's solution. The bath temperature was 36·5° to 37° C.

The rate, amplitude and tone of the frog heart *in situ* were gradually decreased by application of solutions of the drug. Following partial cardiac depression, vagal stimulation did not produce its characteristic effects. Similar effects were noted with the isolated frog's heart perfused through the aorta or sinus venosus with Ringer's solution containing 1 part in 200,000 of the alkaloidal hydrochlorides. There was no evidence of heart block.

The amplitude of the beat of Langendorff preparations of rat, guinea-pig, rabbit and kitten hearts was reduced by 0·5 to 1·0 mg. of the alkaloidal hydrochlorides given as an injection into the cannula. 2 to 3 mg. caused irreversible stoppage of the heart.

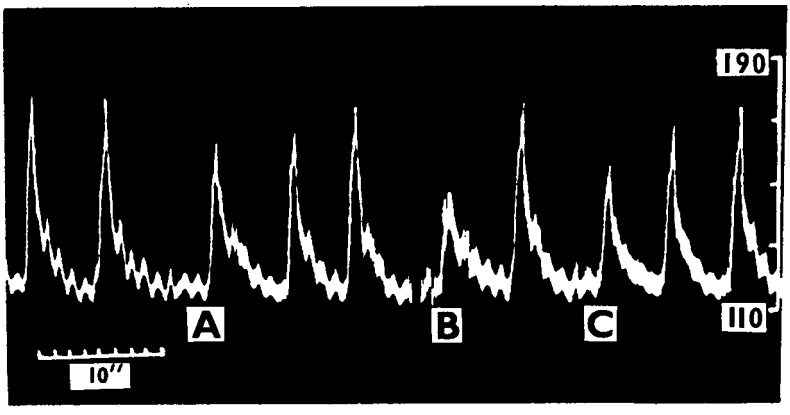

FIG. 1. Female spinal cat, weighing 4 kg. Recording of the arterial blood pressure. All pressor responses are caused by the injection of 3 μg. of adrenaline hydrochloride into the jugular vein. At A the injection was preceded by 2 mg. of the total hydrochlorides of *A. ulei*; at B by 5 mg.; and at C by 3 mg. Time base 10 seconds.

When perfused with Ringer's solution containing 1 part in 200,000 of the hydrochlorides, the effects were similar but if perfusion was prolonged for longer than 10 minutes, the heart stopped irreversibly. The cardiac outflow was reduced from 3·2 ml. per minute to about 1·0 to 1·5 ml. per minute.

There was marked depression of the beat of the isolated rabbit's auricles when 1 mg. of the alkaloidal hydrochlorides was added to the bath. 0·1 to 0·5 mg. caused marked constriction of the blood vessels of the rat hind legs preparation perfused through the abdominal aorta, and of those of the isolated perfused rabbit's ear. No alteration of the responses to 5 μg. of acetylcholine chloride or of 0·01 μg. of adrenaline hydrochloride could be shown.

In the cat anæsthetised with ether and chloralose, administration of small doses of the drug (1·5 mg./kg.) into the jugular vein caused no noticeable effects upon depth or frequency of respiration. The blood

pressure was depressed after each administration. The magnitude of the depression was proportional to the dose of alkaloid given. No reversal of the pressor response to doses of 5 μg. of adrenaline hydrochloride could be shown. There was no antagonism to the depressor responses to 2·0 μg. of histamine acid phosphate or of 2 μg. of acetylcholine chloride. In the spinal cat there was no fall in blood pressure when 1·25 mg./kg. of the drug was administered intravenously, but the pressor response to 3 μg. of adrenaline hydrochloride was inhibited by almost 50 per cent. 2 mg. doses of the alkaloidal hydrochlorides did not protect mice, weighing 20 to 21 g., from the effects of lethal doses of adrenaline hydrochloride.

When tested by the method of Sollman[5] as modified by Bülbring and Wajda[6] a graded local anæsthetic action was shown but this was not as marked as that reported by us[1,2,3], for other Aspidosperma alkaloids. To rule out the possibility of a generalised toxic effect on nervous tissues, or of a neuromuscular block, immediately after sensory paralysis to the highest concentration of the acid, the sciatic nerve was exposed and stimulated, when the gastrocnemius muscle was found to respond normally.

2 mg. of the alkaloids, when injected intraperitoneally into mice weighing 23 to 25 g., caused no appreciable fall in rectal temperature.

Injection of 0·5 mg. of alkaloidal hydrochlorides into the ventral lymph sac of frogs caused stimulation followed by depression (from which there was recovery). 3·0 mg. caused paralysis of the limbs and death in about half of the number of frogs used.

In mice weighing 19 to 20 g. intraperitoneal injection of 3 mg. of the hydrochlorides caused chattering movements of the jaws followed by tonic convulsions, and death of about half of the mice used. The position of the dead animals was characteristic; the back was arched, the tail bent stiffly forwards over the back. The hind limbs were rigid and stretched backwards and in line with the body. The animals which recovered were quiet for a period of several hours.

In rabbits, intravenous injection of 10 mg./kg. caused chattering movements of the jaws, general uneasiness, exaggerated scratching movements of the fore-limbs, salivation and lachrymation, and characteristic arching of the back. Respiration was rapid and deep. After about 20 minutes there was recovery.

No antimalarial activity was shown when the alkaloids at a 2 mg. dose level were tested by the method of Rollo[7], against *Plasmodium berghei* in mice.

The alkaloidal hydrochlorides were amœbicidal *in vitro* at a concentration of 1 in 10,000, but were inactive at a concentration of 1 in 100,000. This is about 1/10 as active *in vitro* as emetine hydrochloride.

Discussion

These alkaloidal hydrochlorides appear to have no generalised anticholinergic properties. The spasmogenic actions of acetylcholine on gut and uterus are antagonised, but antagonism is not evident on

skeletal or cardiac muscle, or on the musculature of the blood vessels. Local anæsthetic activity, associated by many workers[8–15] with anticholinergic properties, is weak. Again there is no evidence of antihistaminic activity nor could we show antagonism to adrenaline in the anæsthetised cat, although antagonism was shown on the spinal animal. The alkaloids appear to exert their effects on frog skeletal muscle by a direct mechanism which is not antagonised by atropine, hexamethonium or *d*-tubocurarine. This does not therefore appear to be a cholinergic effect, although some of the actions on the intact rabbit lead us to consider that the alkaloids might have cholinergic properties of their own. The alkaloids appear to have a direct and marked stimulant action on the central nervous system. The amœbicidal activity is of some interest.

Summary

1. The alkaloids of the bark of *Aspidosperma ulei* antagonised the stimulant action of the acetylcholine on the smooth muscle of the gut and uterus, but not at other sites.
2. A marked central stimulant action was observed in mice and in rabbits.
3. Antagonism to the pressor effects of adrenaline on the spinal cat was shown.
4. *In vitro* amœbicidal activity was shown.

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