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# TRYPTAMINES AND SPINAL CORD REFLEXES IN CATS

BY

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Tryptamine and tryptamine-like compounds have many actions on the central nervous system. The convulsant effects of tryptamine in cats and rats were described by Laidlaw (1912) and Tedeschi, Tedeschi & Fellows (1959) and a head shake or " twitch " has been described in mice (Corne, Pickering & Warner, 1963). The effects of intraventricular injections of 5-hydroxytryptamine and chemical antagonists on behaviour in cats were observed by Feldberg & Sherwood (1954) and by Gaddum & Vogt (1956) and the effects on cerebral electrical activity after systemic or intraventricular injections by Bradley (1958). The actions of 5-hydroxytryptamine on evoked responses in the cat brain were reported by Malcolm (1958) and of a series of indoles on transmission in the cat lateral geniculate nucleus by Evarts (1958). Following the suggestion that the effects of tryptamines on the central nervous system would be better studied by using $\alpha$-methyltryptamine, which is resistant to breakdown by monoamine oxidase (Vane, 1959), tryptamine and $\alpha$-methyltryptamine have been tested on behaviour in rats (Randrup, Munkvad & Udsen, 1963) and on behaviour and cerebral electrical activity in chickens (Dewhurst & Marley, 1965a, b) and cats (Bradley & Marley, 1965). Tryptamine derivatives have been applied iontophoretically to neurones of the spinal cord (Curtis, 1962), the cerebral cortex (Krnjević & Phillis, 1963), the brain stem (Bradley & Wolstencroft, 1965) and the lateral geniculate nucleus (Curtis & Davis, 1962) of the cat. Results of these applications varied according to whether anaesthetic was used and with the location at which they were applied.

The present paper extends the observations of Vane, Collier, Corne, Marley & Bradley (1961) and Marley & Vane (1963) on spinal cord reflexes in cats; the site of action of tryptamine has been partially localized and the effects of amine oxidase inhibitors, reserpine and tryptamine antagonists have been studied. An account of some of the experiments was communicated to the British Pharmacological Society in January, 1961.

## METHODS

Male and female cats were used. Anaesthesia was induced with ethylchloride and ether and the trachea cannulated. The anaesthesia was continued with halothane until the cats were made spinal by destroying the brain through the approach for the *encéphale isolé* described by Bradley & Key (1958). In many of the cats the spinal cord was additionally transected at the junction of the thoracic and lumbar regions. Halothane anaesthesia was also used in those cats which were decerebrated

through an occipital craniotomy. All cats were artificially ventilated. Carotid arterial blood pressure was recorded with a mercury manometer, writing on a kymograph. Intravenous injections were made *via* a cannula in the jugular vein.

*Mechanical recording of reflexes*

*Flexor reflex.* After dividing the ipsilateral ilio-psoas muscle and femoral nerve, a flexor reflex (Liddell & Sherrington, 1929) was elicited by stimulating the central end of the divided posterior tibial nerve; the resulting twitch of the tibialis anterior muscle was recorded with a Brown-Schuster myograph writing on smoked paper. The nerve was stimulated with supra-maximal recta-linear pulses of 0.5 msec duration and a strength of 2–10 V at rates between 8–12 min or for tetanic stimulation at 10/sec for 5 sec.

*Crossed extensor reflex.* A crossed extensor reflex from the other leg was sometimes recorded as well as the flexor reflex. Both lower limbs were fixed by drills through the femurs. For the crossed extensor reflex, the contralateral ilio-psoas was divided and the contralateral quadriceps femoris mobilized by disconnecting the patellar tendon from the tibia and attaching it to a Brown-Schuster myograph. The saphenous nerve and the nerve to the sartorius were divided but that to the quadriceps femoris was preserved. The contralateral reflex was elicited by stimulating the central end of the divided ipsilateral posterior tibial nerve.

*Reflex inhibition.* Reflex contraction of the quadriceps femoris was elicited by stimulating the central end of the contralateral divided sciatic nerve at 20/sec for 90 sec (see above). During this stimulation the central end of the divided ipsilateral sciatic nerve was excited for 30 sec at 50/sec; this inhibited the contraction of the quadriceps femoris, but the contraction returned immediately after stopping stimulation of the ipsilateral sciatic nerve and was maintained for the rest of the 30 sec period during which the contralateral sciatic nerve was stimulated.

*Neuromuscular transmission*

Records of the twitch from the tibialis anterior and the soleus muscle were obtained with a Brown Schuster myograph in response to stimulation of the peripheral end of the divided sciatic nerve at a rate of 6 to 12/min.

*Electromyograms*

The recording electrodes were made from 50 cm lengths of Diamel-coated silver wire, 0.3 mm in diameter, prepared as described by Key & Marley (1961). They were inserted into the tibialis anterior muscle and activity recorded on a Kaiser 8-channel electroencephalograph.

*Electrical recording of evoked potentials*

The appropriate vertebral laminae were removed and the lumbar and sacral spinal nerve roots sectioned. Stimulating electrodes were placed on a dorsal root and recording electrodes on the corresponding ventral root (Lloyd, 1943). The cord was divided distal to the roots used for stimulating and recording. Decamethonium (50 $\mu$g/kg intravenously repeated when necessary) was injected to prevent movements of the cat from interfering with recording. The dorsal roots were stimulated with supramaximal rectangular pulses of 0.5 msec duration at a frequency of 10/min.

*Intra-arterial injections*

Tryptamines were injected into the aorta so that little or none of the drug reached the limb under test. This was achieved in two ways (Fig. 1): (a) in eviscerated cats given heparin (10 mg/kg) part of the aortic blood was diverted through polyethylene tubing from the cannulated stump of the coeliac artery (C.A.) into the common iliac artery (C.I.) on the side from which the flexor reflex was being elicited. A renal artery was cannulated for injection and the other was tied, as were both common iliac and the median sacral arteries. Thus, drugs injected through the cannula in the renal artery reached only the spinal cord. (b) A catheter was passed *via* a common iliac artery into the aorta. During injections through this catheter, the other common iliac artery was occluded by a clip so that the drug passed directly to the spinal cord but not to the leg.

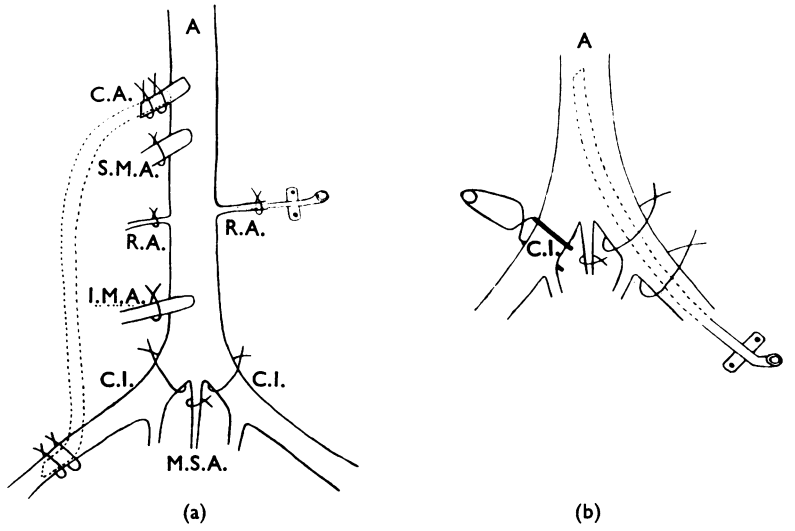


Fig. 1. Circulation for intra-arterial injections. The left hand figure shows the abdominal aorta (A) and vessels from ventral aspect in an eviscerated cat. Arterial blood passed from the coeliac artery (CA) through polyethylene tubing (dotted lines) to the common iliac artery (CI) of the limb from which the flexor reflex was elicited. Both common iliac arteries were ligated. The superior mesenteric (SMA), inferior mesenteric (IMA), renal (RA) and median sacral (MSA) arteries were ligated. An injection cannula was tied into a renal artery. The right hand figure shows an alternative arrangement. An injection cannula was passed retrogradely into one common iliac artery and tied with its tip in the aorta.. The contralateral common iliac artery of the limb from which the flexor was elicited was clipped during the injection.

*Intraduodenal injections*

For intraduodenal injection of L-tryptophan and DL-5-hydroxytryptophan, a midline abdominal incision was made and a polyvinyl tube was passed through an incision in the greater curvature of the stomach into the duodenum. The tube was tied with the tip distal to the pyloric sphincter, the abdomen closed, and the free end of the catheter brought to the exterior.

*Drugs*

Those used included the hydrochlorides of cocaine, dopamine, nialamide, β-phenethylamine, pheniprazine, (±)-β-hydroxyphenethylamine, (+)-α-methyltryptamine, 5-methoxytryptamine, (±)-5-methoxy α-methyltryptamine, N,N-dimethyltryptamine, phenoxybenzamine, strychnine, tyramine and tryptamine. In addition, decamethonium iodide, indol-3-ylacetic acid, 5-hydroxy- and 6-hydroxy-tryptamine creatinine sulphate, α-methylbenzyl hydrazine oxalate (mebanazine), methysergide, (−)-noradrenaline hydrogen tartrate, N-acetyl 5-hydroxytryptamine, phenelzine hydrogen sulphate, reserpine, DL-5-hydroxytryptophan and L-tryptophan were tested. Doses of salts are given in terms of the base. Injections were intravenous unless otherwise stated.

## RESULTS

### *Neuromuscular junction*

In 2 cats twitches of the soleus and tibialis muscles were simultaneously elicited by electrical excitation of the peripheral end of a divided sciatic nerve. Tryptamine (2 mg/kg) and α-methyltryptamine (2 mg/kg) injected retrogradely through the contralateral iliac artery into the aorta (as in Fig. 1, but without clamping the iliac artery) or intra-

venously, did not alter twitch tensions of the two muscles. Even with larger doses (5 mg/kg intravenously) there was no effect on the neuromuscular junction.

*Flexor reflex*

Tryptamine increased the twitch tension of the reflexly excited anterior tibialis muscle in the spinal cat (Fig. 2). With a small dose (0.25 mg/kg) only peak tension was increased

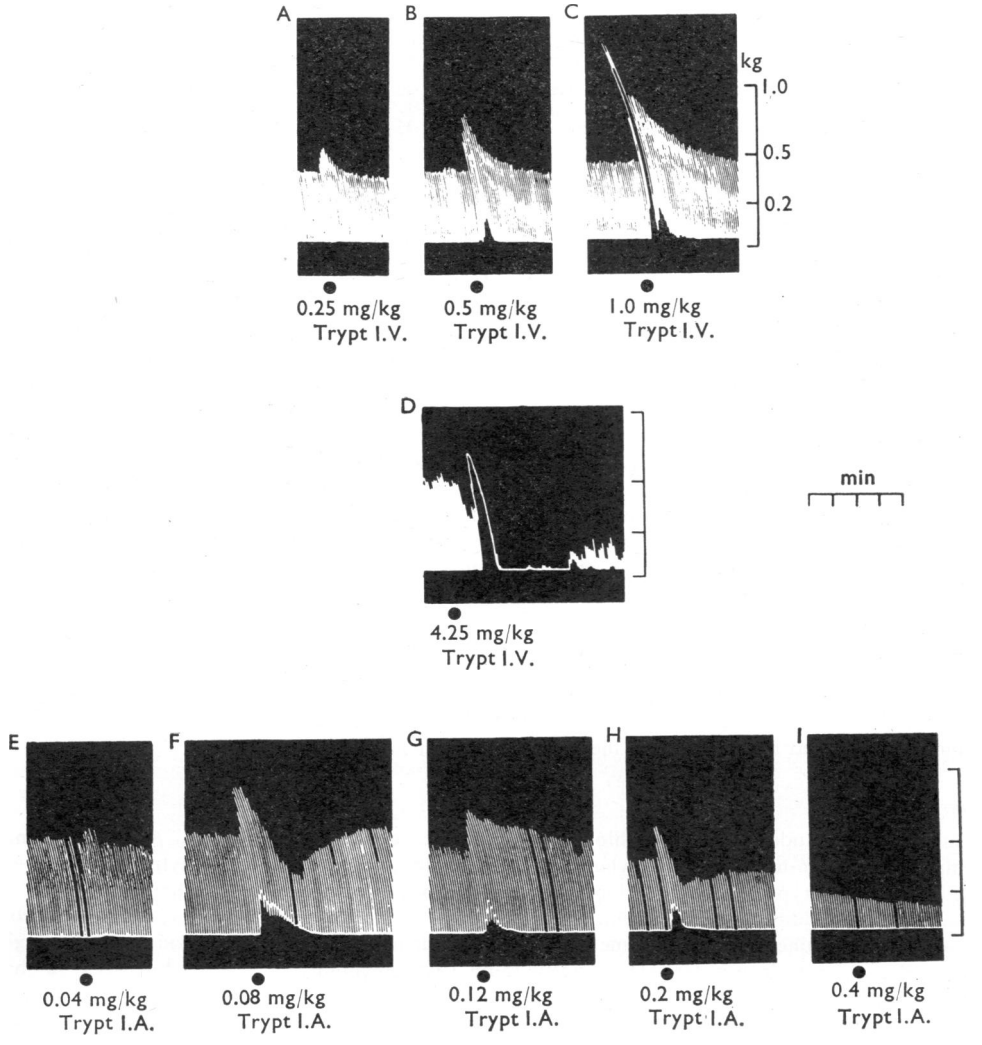


**Fig. 2. Flexor reflex. In this and subsequent figures, the flexor reflex was evoked by stimulating the central end of the divided posterior tibial nerve and recording twitches from the ipsilateral tibialis anterior muscle. A–D, spinal cat (2.3 kg). When tryptamine (A–C, 0.25, 0.5 and 1 mg/kg) was injected intravenously, the reflex was enhanced. After tryptamine (D, 4.25 mg/kg intravenously) there was a contracture and temporary abolition of twitch. E–I, another spinal cat 2.8 kg). Intra-arterial injections (as in Fig. 1B) of tryptamine (0.08 and 0.12 mg/kg) enhanced the flexor reflex, but after 0.2 mg/kg there was a depression following the enhancement. Time in min ; vertical scales in kg tension as in subsequent tracings.**

(Fig. 2A). With a larger dose (0.5 mg/kg) basal and peak tension were augmented (Fig. 2B) and with 1 mg/kg (Fig. 2C) the peak tension developed was more than double that of the control. Larger doses (4.25 mg/kg, Fig. 2D) gave a different response; after the increase in tension the twitch was depressed and sometimes absent for several minutes and much reduced when it returned (Fig. 2D). At this stage, the twitch was not restored by increasing the intensity or duration of shock, nor by further intravenous doses of tryptamine. The twitch was, however, partially or wholly restored by strychnine (20 to 80 $\mu$g/kg). The last part of the figure illustrates another experiment in which tryptamine was given intra-arterially. Similar effects were obtained with much smaller doses; whereas tryptamine (0.04–0.12 mg/kg intra-arterially) gave mainly an increase in twitch tension, 0.2–0.4 mg/kg reduced the twitch tension. Again, the tension was not restored by increasing the stimulus strength or duration. The dual nature of the tryptamine effect after either intravenous or intra-arterial doses made it impossible to obtain a consistent dose/response curve.

The effects of tryptamine on the individual twitches are shown in Fig. 3. Tryptamine (1 mg/kg) greatly prolonged the twitch duration despite a small increase of peak tension.

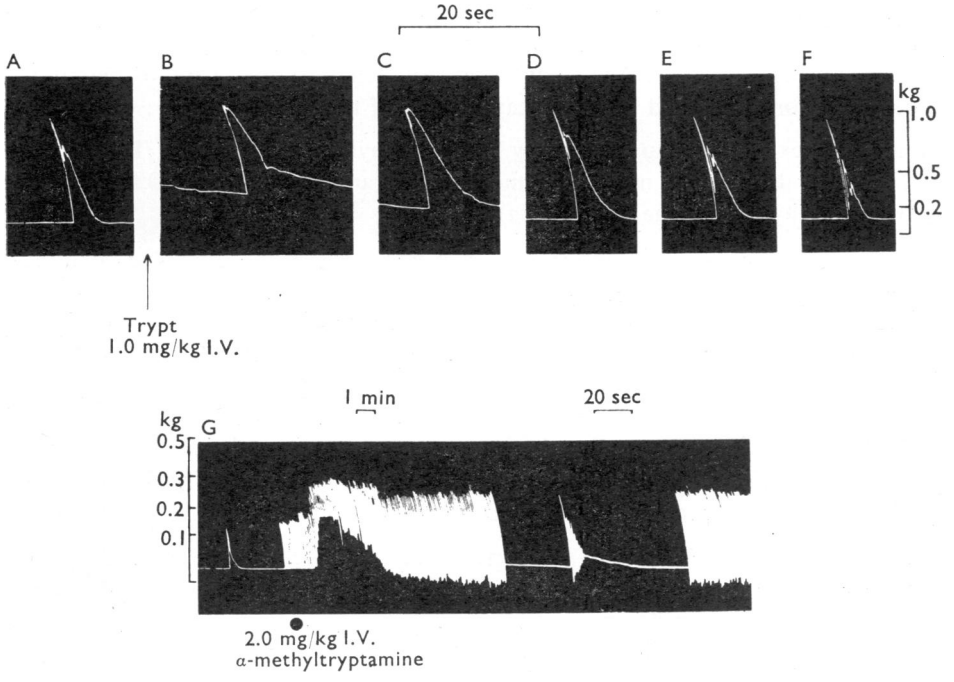


Fig. 3. The flexor reflex was elicited in a spinal cat (3.5 kg) with additional thoracico-lumbar cord transection. Responses to individual shocks were recorded on a fast drum. A, control. B, C, D, E and F, at 1.5, 2, 2.5, 4.5 and 6 min respectively after tryptamine (1 mg/kg) showing increased twitch duration, peak tension and basal tension. G, another spinal cat (3 kg) with additional thoracico-lumbar cord transection. $\alpha$-Methyltryptamine (2 mg/kg) increased the basal tension for 4 min and there was also a longer-lasting increase in peak tension. The twitch duration was prolonged with oscillations on decaying part of twitch, shown for one twitch at a faster drum speed in the middle of the trace, as compared with the one at the beginning.

The control twitch duration was 1.5 sec. At 1.5, 2, 2.5, 4.5 and 6 min after the injection the twitch durations were 18.8, 9.5, 7.0, 4.7 and 3.5 sec (Fig. 3, A–F). The twitch responses in B and C are not completely shown. Since the nerve was stimulated at a frequency of 12/min, the increased basal tension after tryptamine was partly ascribable to successive shocks reaching the tibialis anterior before relaxation was complete. In addition, another factor contributed to the increased basal tension evoked by tryptamine. This is illustrated later (Fig. 5) and is due to the development of tension in the tibialis anterior after tryptamine *in the absence of stimulation of the posterior tibial nerve.* This tension developed more gradually and was smaller than when the nerve was being stimulated. The difference was presumably due in the latter case to recruitment of spinal neurones in addition to those excited by the drug. The effect of tryptamine on basal tension with or without nerve stimulation was abolished by dividing the anterior tibial nerve.

The effects of $\alpha$-methyltryptamine (2 mg/kg) are shown in Fig. 3G. As with tryptamine, an increase of basal and peak tensions developed within 1 min of the injection. Basal tension was elevated for 4 min, at least twice as long as the increase with comparable doses of tryptamine (compare Fig. 3G with 9C). The response differed from that due to tryptamine in that peak tension was elevated for the remainder of the experiment. The effect of $\alpha$-methyltryptamine on the single twitch was seen more clearly by recording with a faster drum speed; twitch tension and twitch duration were increased and a series of oscillations appeared on the decaying part of the twitch.

The flexor reflex was also augmented by N,N-dimethyltryptamine (2 mg/kg), 5-hydroxytryptamine (0.5, 1.0 and 2 mg/kg), 5-methoxytryptamine (0.25 and 0.5 mg/kg) and 5-methoxy $\alpha$-methyltryptamine (0.4 mg/kg).

*Relation to changes in blood pressure.* The effects of tryptamine and of $\alpha$-methyltryptamine on the reflexly-induced tibialis twitch were not dependent on changes in blood pressure, as is evident from Fig. 4. Noradrenaline (5 $\mu$g/kg) and tyramine (2 mg/kg) produced rises in arterial pressure of 120 and 150 mm Hg respectively without significant alteration in peak or basal tensions of the flexor reflex. Tryptamine (1 mg/kg) and $\alpha$-methyltryptamine (0.5 mg/kg) elicited smaller increases in carotid arterial pressure of 100 and 90 mm Hg respectively, but in contrast, peak and basal tensions of the flexor reflex were augmented. The increases in peak tension with $\alpha$-methyltryptamine persisted when its effect on blood pressure had abated (Fig. 4D).

*Metabolites of tryptamine and 5-hydroxytryptamine.* Metabolites of tryptamine and 5-hydroxytryptamine were tested to see whether the after-depression of the flexor reflex, particularly with the larger doses of tryptamines, was due to breakdown of the parent substances into their metabolites.

Those tested included indol-3-ylacetic acid, 5-methoxytryptamine and N-acetyl-5-hydroxytryptamine: 6-hydroxytryptamine was also examined, since according to Jepson, Zaltman & Udenfriend (1962) tryptamine and related indoles are metabolized by 6-hydroxylation. Peak and basal tensions were raised by 5-hydroxy- and even more so by 5-methoxytryptamine (0.25 mg/kg) in experiments in which peak tension only was increased by tryptamine (0.25 mg/kg); indeed, 5-methoxytryptamine was the most potent of all compounds tested, including tryptamine and $\alpha$-methyltryptamine. In contrast,

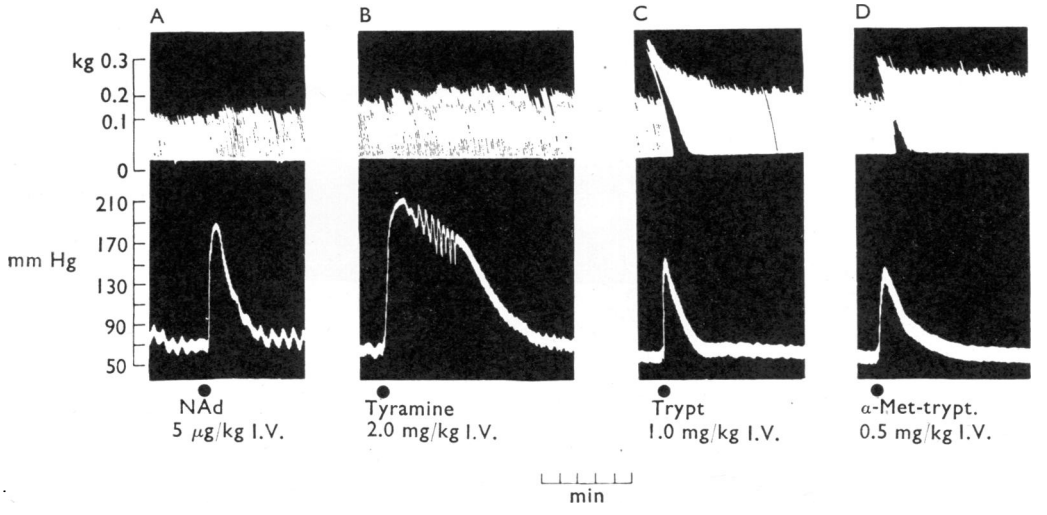


Fig. 4. The flexor reflex and blood pressure were recorded in a spinal cat (2 kg) with additional transection of the cord at the thoracico-lumbar junction. The first two tracings show large pressor effects with noradrenaline (A, 5 μg/kg) and tyramine (B, 2 mg/kg) with no change in the reflex. Smaller pressor effects were obtained with tryptamine (C, 1 mg/kg) and α-methyl-tryptamine (D, 0.5 mg/kg), but there was a substantial increase in peak and basal twitch tensions.

indole-3-ylacetic acid (0.25 and 2 mg/kg), N-acetyl 5-hydroxytryptamine (0.25 and 2 mg/kg) and 6-hydroxytryptamine (0.25 and 0.5 mg/kg) were without effect on the reflex. Mebanazine (20 mg/kg intradermally) was then injected and the metabolites tested after 120 min. N-acetyl 5-hydroxytryptamine (0.25 and 2 mg/kg), 6-hydroxytryptamine (0.25 and 0.5 mg/kg) and indole-3-ylacetic acid (2 mg/kg) were still without effect on the twitch. However, indole-3-ylacetic acid (6 mg/kg) produced after 4 min a small increase in peak and basal tensions.

*Potentiation.* Since tryptamine is rapidly inactivated by monoamine-oxidase (Weissbach, Lovenberg, Redfield & Udenfriend, 1961), its effect was tested after pretreating cats with an amine-oxidase inhibitor. Figure 5A shows the effect of tryptamine (1 mg/kg) on basal tension of the tibialis anterior in the absence of nerve stimulation; tension developed to a maximum of 0.1 kg lasting 1 min. Ninety minutes after injecting mebanazine (10 mg/kg), tryptamine (1 mg/kg) was again injected; tension increased to a maximum of 0.25 kg fluctuating in intensity over 5 min (Fig. 5B). The effect of tryptamine on electromyographic activity was similarly potentiated by mebanazine.

The effect of tryptamine on the flexor reflex was also enhanced by pretreatment with an amine oxidase inhibitor. Responses to tryptamine (0.25 mg/kg, Fig. 5C) were greatly enhanced 45 min after mebanazine (10 mg/kg, Fig. 5E). Similar results were obtained with tryptamine in another cat given mebanazine, and in a cat pretreated with a hydrazide monoamine-oxidase inhibitor, nialamide (15 mg/kg, 60 min previously). Suppression of twitch was still obtained with larger doses of tryptamine in cats pretreated with a single dose of amine oxidase inhibitor. However in another two cats pretreated more extensively with mebanazine (20 mg/kg intraperitoneally 24, 12 and 1 hr previously) and

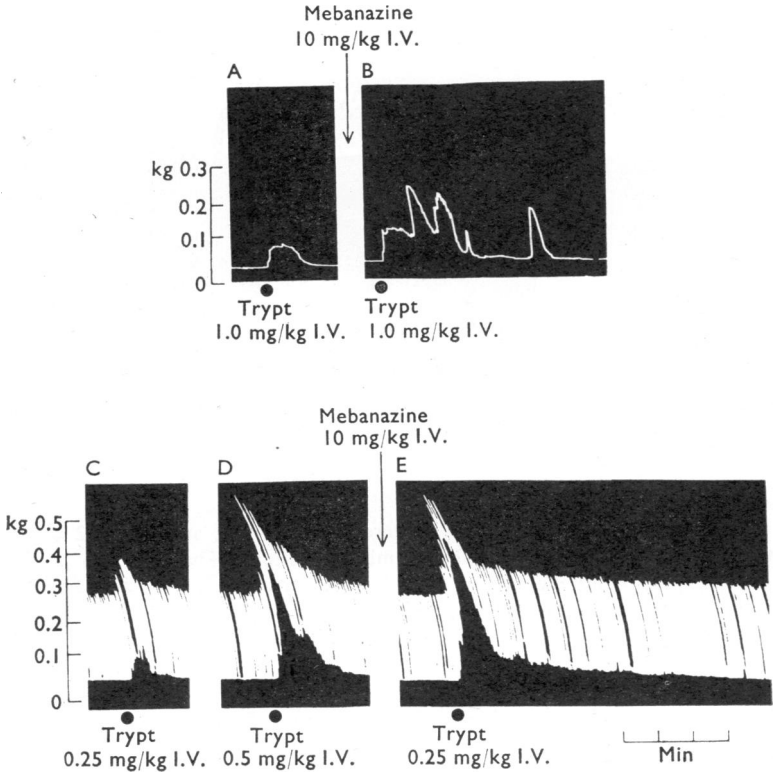


Fig. 5. Potentiation of tryptamine by mebanazine. A, B, spinal cat (2.4 kg) with additional thoracico-lumbar cord transection. Tone of innervated tibialis anterior recorded myographically. An increase of tone produced by tryptamine (A, 1 mg/kg) was much increased after mebanazine (B, 10 mg/kg, 90 min previously). C–E, another spinal cat (4 kg) with additional thoracico-lumbar cord transection: the tracing showing an increase in peak and basal twitch tensions due to tryptamine (C, 0.25 and D, 0.5 mg/kg) with potentiation (E) of the smaller dose after mebanazine (10 mg/kg, 45 min previously).

then made spinal, tryptamine in repeated doses to a total of 30 mg/kg intravenously raised basal and peak tensions of the flexor reflex, a change which persisted for the duration of the experiment (2 hr). Since, in untreated cats, one-sixth of dose would have suppressed the twitch it would appear that the suppression may be due to metabolites.

*Other amines.* Some amines normally ineffective on the flexor reflex were tested in a cat pretreated with mebanazine (10 mg/kg 90 min previously). $\beta$-phenethylamine (1 mg/kg) now increased peak and basal twitch tensions; $\beta$-hydroxyphenethylamine (1 mg/kg), tyramine (1 mg/kg), dopamine (1 mg/kg) and noradrenaline (10 $\mu$g/kg) remained ineffective.

*Antagonist at tryptamine receptors.* Methysergide, a potent antagonist at tryptamine receptors (Doepfner & Cerletti, 1958) prevented the effects of tryptamine on the spinal reflex (Fig. 6). Tryptamine (2 and 1 mg/kg) brought about the usual increase in basal

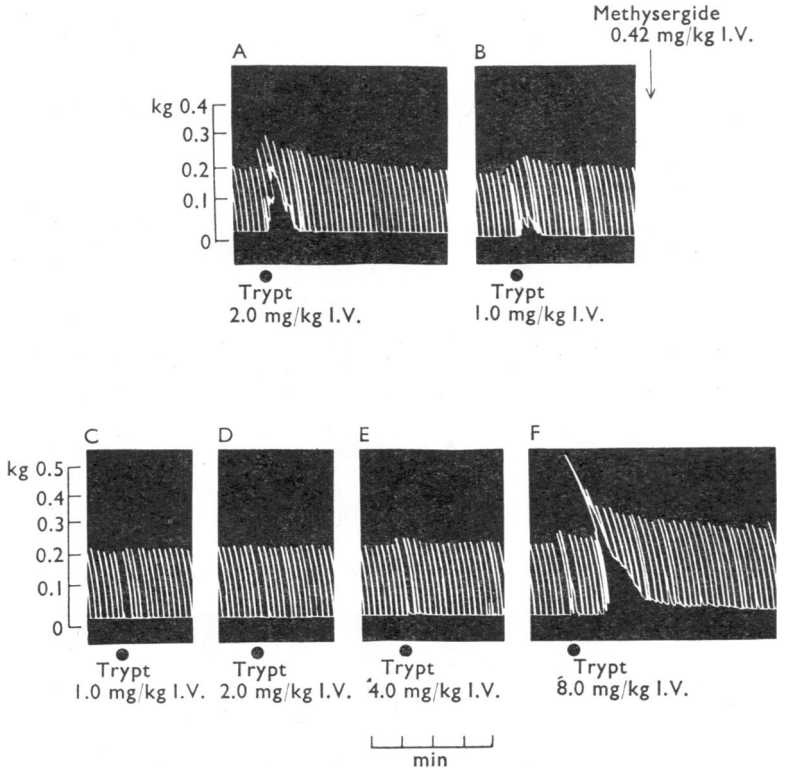


Fig. 6. The flexor reflex was recorded in a spinal cat (2.3 kg) with additional spinal transection at thoracico-lumbar junction. Control responses were obtained to tryptamine (A, 2 and B, 1 mg/kg). After methysergide (0.42 mg/kg) both doses of tryptamine (C, D) were ineffective, but tryptamine (E, 4 mg/kg) produced a small increase of peak and basal twitch tensions. The antagonism was surmounted by tryptamine, 8 mg/kg (F). Note the negligible increase of peak and basal tensions at time of injection but a much greater increase of twitch tensions 90 sec later.

and twitch tension of the flexor reflex, but after methysergide (0.42 mg/kg) these doses were without effect; tryptamine (4 mg/kg) evoked only a diminutive increase in peak and basal tensions, but the antagonism was surmounted by 8 mg/kg. This response, however, was unusual in two ways. First, although there was an immediate small increase in peak tension, there was a delay of 90 sec before peak and basal tensions were markedly raised; secondly, the peak tension remained raised after the injection whereas, in a cat not given methysergide, the contracture elicited by this large dose of tryptamine would have been followed immediately by abolition of twitch. These results were confirmed in two other cats.

*Other antagonists.* Phenoxybenzamine in sufficient dose (2 mg/kg) to abolish the pressor actions of noradrenaline (10 $\mu$g/kg) did not modify the effects of tryptamine (0.5 and 1 mg/kg) on the flexor reflex. Similarly cocaine (2 mg/kg) which abolished the pressor actions of tyramine (0.5 mg/kg) did not alter the response of the flexor reflex to tryptamine (0.5 and 1 mg/kg) or to $\alpha$-methyltryptamine (1 mg/kg).

*Chronic treatment with reserpine.* In cats treated chronically with reserpine, reflex flexor twitch tension was low with a peak of less than 0.1 kg. Tyramine (2 mg/kg) was injected intravenously and reserpinization only considered adequate if it had little or no pressor action (Fig. 7A). For details of reserpine dosage, see the legend to Fig. 7.

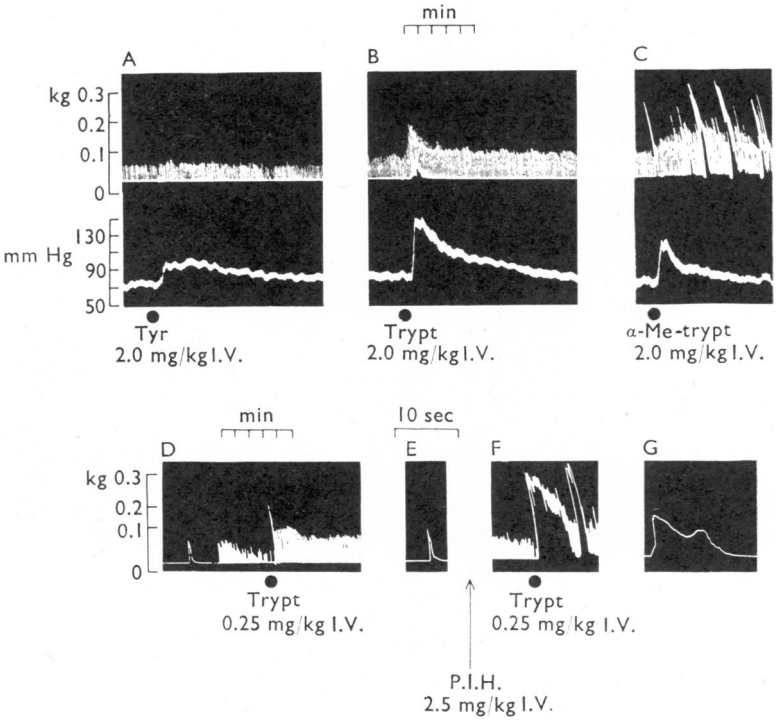


Fig. 7. The flexor reflex and blood pressure were recorded in 2 cats which had been pretreated with reserpine. A–C, spinal cat (1.5 kg) given reserpine (1.75 mg/kg intraperitoneally, 48 hr previously and 1 mg/kg intraperitoneally 24 hr previously). A, control with greatly reduced reflex twitch tension. The pressor effect of tyramine (2 mg/kg) was also much reduced. However, there was an increase in peak and basal tensions after tryptamine (B, 2 mg/kg) and α-methyltryptamine (C, 2 mg/kg). D–G, another spinal cat (2.8 kg) given reserpine (0.5 mg/kg intraperitoneally 48 hr previously and 1 mg/kg intraperitoneally 24 hr previously). D, control, with much diminished twitch tension shown initially on a fast drum. The peak tension was increased by tryptamine (0.25 mg/kg intravenously), although the twitch duration was little altered (compare E with D). The increase in twitch tension induced by tryptamine (0.25 mg/kg) was much enhanced and prolonged (F, G) after pheniprazine (P.I.H. 2.5 mg/kg).

Tryptamine and particularly α-methyltryptamine increased peak and basal twitch tensions (Fig. 7, B, C) in spite of chronic reserpine treatment. In another experiment (Fig. 7, D, E) on a chronically reserpinized spinal cat peak tension was doubled following tryptamine (0.25 mg/kg). Potentiation of the effects of tryptamine by pretreatment with an amine oxidase inhibitor was also obtained in chronically reserpinized cats. The increase in peak and basal tensions and in duration of the single flexor twitches produced by tryptamine (0.25 mg/kg) were considerably augmented 17 min after a dose of

pheniprazine (2.5 mg/kg), which by itself had no effect on twitch tension (compare Fig. 7E and G).

*Amino-acid precursors of tryptamine and 5-hydroxytryptamine.* L-tryptophan or DL-5-hydroxytryptophan injected intraduodenally (25 mg/kg) or intravenously (5 mg/kg) had no effect on the flexor reflex. Four cats were pretreated with an amine oxidase inhibitor to reduce or prevent metabolism of any tryptamine and 5-hydroxytryptamine formed. The first 2 cats were given mebanazine (20 mg/kg) intraduodenally 120 min previously. In the one cat peak twitch tension was increased and twitch duration prolonged within 19 min of tryptophan (25 mg/kg) injected intraduodenally (compare Fig. 8B and D). Although these changes developed gradually, occasional twitches manifested greater peak or basal tension (Fig. 8D and E) than the remainder. These effects subsided after 40 min, but even though twitch tension had declined to below that of the control, excitability was still enhanced as evident from a larger reflex response on tetanizing the posterior tibial nerve (Fig. 8G). Similar effects were observed in a second cat.

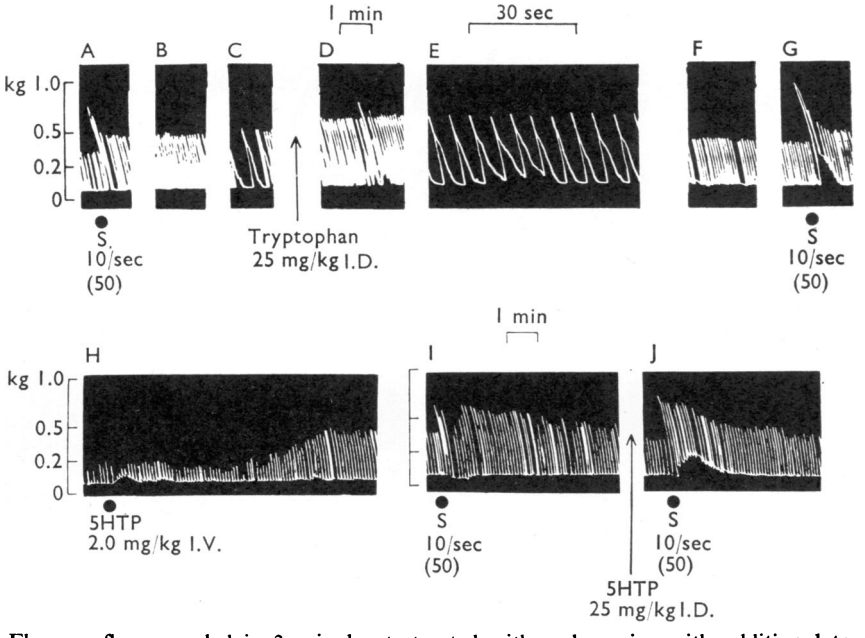


Fig. 8. Flexor reflex recorded in 3 spinal cats treated with mebanazine with additional transection of the spinal cord at the thoracico-lumbar junction. A to G, spinal cat (2 kg). A, control response to tetanus (S) superimposed on responses to single shocks at 12/min. B and C, control responses recorded on a slow and fast moving drum to single shock to the nerve. Between C and D, mebanazine (20 mg/kg intraduodenally) and tryptophan (25 mg/kg intraduodenally) given 120 min later. D, enhanced basal and peak twitch tensions 19 min after tryptophan. The intermittent enhanced basal tension is shown better in E on a fast-moving drum. F, reduced peak tension 40 min after tryptophan, although (G) response to tetanus still enhanced. H, spinal cat (2.5 kg) and I, J (spinal cat, 2.2 kg) given mebanazine (20 mg/kg intraperitoneally 24 and 12 hr previously and 20 mg intraduodenally 1 hr before). H, enhanced peak tension developing 5 min after 5-hydroxytryptophan (2 mg/kg intravenously). I, control response to tetanizing (S) posterior tibial nerve. J, peak tension unaltered by 5-hydroxytryptophan (25 mg/kg intraduodenally) but response to tetanus increased.

The other 2 cats had longer pretreatment with mebanazine (20 mg/kg intraperitoneally, 24 and 12 hr previously and 20 mg/kg intraduodenally, 1 hr before). In the first (Fig. 8H), DL-5-hydroxytryptophan (20 mg/kg) gradually increased peak tension after a delay of 5 min. In the other cat (Fig. 8, J) 5-hydroxytryptophan (25 mg/kg intraduodenally) did not alter peak tension or twitch duration but the effects of a tetanus (50 shocks at 10/sec) were enhanced with increase of basal and peak tensions.

*Crossed extensor reflex*

The crossed extensor response of the contralateral quadriceps femoris was recorded at the same time as the flexor response of the tibialis anterior in 6 spinal and in 6 decerebrate cats. Flexor tone predominates in spinal cats and twitches to single shocks and contracture of the tibialis anterior on tetanizing the posterior tibial nerve were readily evoked without response of the contralateral quadriceps femoris (Fig. 9, A, B, C). However, after tryptamine (1 and 2 mg/kg) (Fig. 9 A, C) there was a small increase in

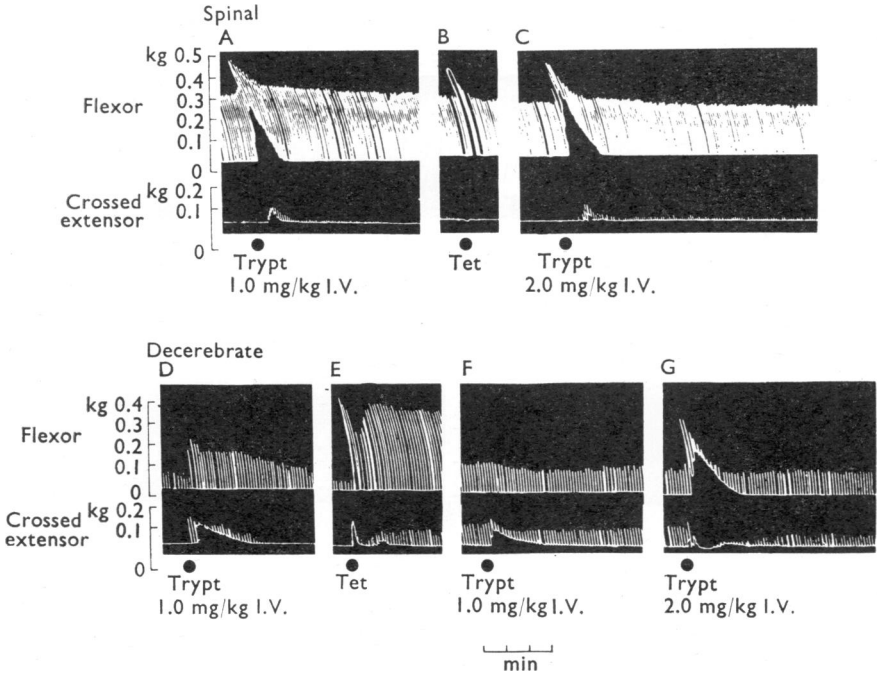


**Fig. 9. Flexor and crossed extensor reflexes in two cats. In both cats the ipsilateral flexor and contralateral extensor reflexes were elicited by stimulating the central end of the divided posterior tibial nerve. A–C, spinal cat (3.2 kg). Tryptamine (A, 1 mg/kg ; C, 2 mg/kg) had a considerably greater effect on the flexor than on the crossed-extensor reflex. A tetanus (B, 200 shocks at 10/sec) to the central end of divided posterior tibial nerve elicited a contracture of the flexor muscle but did not affect crossed extensor. D–G, decerebrate cat (3 kg). Tryptamine (D, 1 mg/kg) enhanced flexor peak tension and extensor peak and basal tensions. A tetanus (E, 200 shocks at 10/sec) to the posterior tibial nerve also increased flexor and extensor tensions. Later there was a reduction of peak tension in flexor and increase in extensor peak and basal tensions with tryptamine (F, 1 mg/kg) but a bigger dose of tryptamine (G, 2 mg/kg) increased flexor peak and basal tensions and reduced extensor twitch.**

basal extensor tension and tiny twitch responses developed. These twitches remained for about 5 min after the smaller dose and about 10 min after the bigger one.

Extensor tone is much more evident in decerebrate cats and a tetanus to the central end of the divided posterior tibial nerve, increased twitches of both the flexor and the crossed extensor responses (Fig. 9E). The twitches of the flexor tibialis anterior dwindled again over the following 60 min, although those of the extensor muscle slightly increased (Fig. 9F). Tryptamine (1 mg/kg) before the tetanus increased the ipsilateral flexor and contralateral extensor twitch responses (Fig. 9D). In 3 of the 6 decerebrate cats, however, the response changed and later in the experiment tryptamine (1 mg/kg, Fig. 9F) increased basal and peak twitch tensions in the crossed extensor muscle but diminished peak twitch tension in the flexor muscle. A larger dose of tryptamine (2 mg/kg), enhanced basal and peak tension of the flexor but simultaneously suppressed twitches of the crossed extensor muscle (Fig. 9G).

Tone in the contralateral quadriceps femoris was also increased by tryptamine (1 and 2 mg/kg) in the absence of nerve stimulation.

*Reflex inhibition of the crossed extensor reflex.* Experiments were made in 6 decerebrated cats. Reflex contraction of the quadriceps femoris muscle was elicited by tetanizing the contralateral sciatic nerve. Once this contraction was at its peak and sustained and while still stimulating the contralateral sciatic nerve, the ipsilateral sciatic nerve was tetanized causing immediate relaxation (inhibition) of the quadriceps femoris. On ceasing stimulation of the ipsilateral nerve, the contraction returned and was increased (rebound). As described by Owen & Sherrington (1911), inhibition of the reflexly contracted contralateral quadriceps by ipsilateral sciatic nerve stimulation was converted to contraction by strychnine (100 $\mu$g/kg). No such reversal was observed after $\alpha$-methyltryptamine in cumulative doses to 20 mg/kg, although rebound was greatly augmented.

*Electromyographic activity*

This was recorded from the tibialis anterior and quadriceps femoris muscles, the tendons of which were attached to myograph plates. Tryptamine (1 mg/kg) increased the amplitude of electromyographic activity from a control of 20 $\mu$V to between 60 and 100 $\mu$V, the duration of which corresponded to the period of increased basal tension shown myographically. Similar but more prolonged effects were elicited by $\alpha$-methyltryptamine (0.5 mg/kg). These effects were not obtained when the nerves connecting the muscles to the spinal cord were divided.

*Spinal cord potentials*

The primary and secondary potentials elicited on electrical stimulation of the dorsal root and recorded from the corresponding ventral root are shown in Fig. 10. Tryptamine (1 mg/kg) increased the primary potential (Fig. 10B) and with a dose of 2 mg/kg the potential was almost double the amplitude of the control (Fig. 10E). The amplitude of primary potential returned to about the control value after the smaller dose of trypamine (Fig. 10C), but was reduced to half (Fig. 10F) after tryptamine (2 mg/kg). Thus, after tryptamine, the electrical events appeared to correspond with those observed with mechanical recording. The changes observed after $\alpha$-methyltryptamine were more

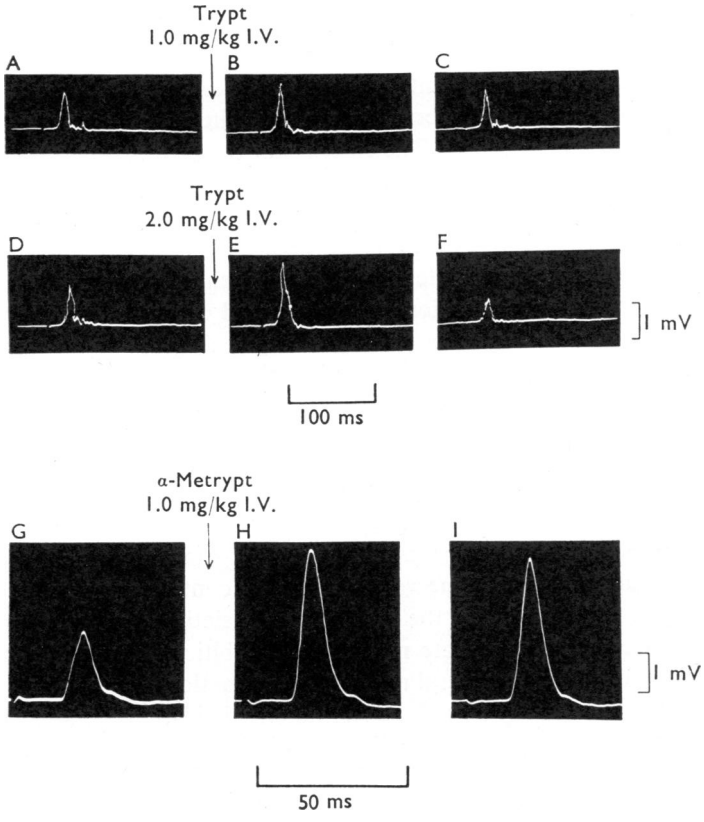


Fig. 10. Potentials recorded from the divided ventral 7th lumbar root on stimulation of the corresponding dorsal root. A to F, spinal cat (2.7 kg) and G to I, another spinal cat (2.5 kg), both with additional thoracico-lumbar cord section and with the femoral and sciatic nerves divided. A and D, controls. B and E, increase in amplitude of potentials 30 sec after tryptamine 1 and 2 mg/kg respectively. C and F, 3 min after tryptamine, showing return to control amplitude in C but diminished amplitude of potentials after the bigger dose in F. G, control. H and I, increase in amplitude of potentials 1 and 35 min respectively after α-methyltryptamine (1 mg/kg). Vertical scales 1 mV ; times 50 and 100 msec.

prolonged. The doubling in amplitude of the primary potential produced by α-methyltryptamine (1 mg/kg) is shown in Fig. 10H and the persistence of the increase illustrated in Fig. 10I recorded 35 min after injection. Again, these electrical events fitted well with the pattern of mechanical response seen after α-methyltryptamine.

## DISCUSSION

The effects of tryptamine were not due to an action at the neuromuscular junction, since the increase in tension of the unstimulated tibialis muscle due to tryptamine was abolished by cutting the anterior tibial nerve, and tryptamine had no effect on the muscle twitch induced by stimulating the peripheral end of the divided sciatic nerve. Nor were the effects likely to be due to impulses reaching the spinal cord through excitation of sensory

nerve endings in lower limb muscles, since injections of tryptamine which did not reach the hind limbs but were distributed mainly to the spinal cord had much greater effects than intravenous injections. The lack of effects of tyramine and of catecholamines in doses sufficient to induce large pressor effects show that tryptamine was not acting through direct or indirect cardiovascular effects. The lack of antagonism by adrenaline receptor antagonists suggested that the effects of tryptamine were neither directly on catecholamine receptors, nor through the release of catecholamines. Supportive evidence was obtained for this from the chronic reserpine experiments.

Antagonism of the tryptamine effects by a specific tryptamine antagonist, methysergide, pointed to an action directly on tryptamine receptors and potentiation of the excitant effects of tryptamine by amine oxidase inhibitors suggested that this action on tryptamine receptors within the spinal cord was being limited in normal conditions by the enzymic breakdown of tryptamine. The results with other tryptamines also confirm an activity on tryptamine receptors. Alpha-methyltryptamine, a type of compound not destroyed by amine-oxidase (Blaschko, 1952) had a much longer action than tryptamine. The fact that 5-substituted compounds which were able to pass the blood-brain barrier (such as 5-methoxytryptamine) were more potent than tryptamine suggested that the receptors were similar to those in smooth muscle (Vane, 1959 ; Handschumacher & Vane, 1967).

That tryptamines have an action on spinal neurones was confirmed by experiments in which evoked potentials in the spinal cord were substantially increased. The spike potential was doubled in amplitude and its duration increased to such an extent that the spike spread into and obscured the small succeeding potentials which represented responses of the same motoneurones to stimuli arriving *via* internuncial neurones (Lloyd, 1943). It is reasonable to assume that these small polysynaptic potentials would also have increased, since we always observed increases in twitch tension in the polysynaptic flexor and crossed extensor reflexes. An increase in amplitude of the primary evoked potential after 5-hydroxytryptophan and tryptophan has also been observed in cats (Anderson & Shibuya, 1966).

The failure of tryptamines to reverse reflex inhibition of the crossed extensor reflex suggested that their action was unlike strychnine, which selectively antagonizes the inhibitory transmitter (Bradley, Easton & Eccles, 1953). Thus, the facilitations of the flexor and crossed extensor reflexes induced by tryptamines were presumably due to recruitment of spinal motoneurones. This recruitment may be due to a pre- or post-synaptic action on any of the spinal neurones that subserve or impinge on these reflex pathways. It is difficult from our experiments to localize the actions of tryptamines any further. The crucial localization would be with iontophoretic application of tryptamines to spinal cord neurones. However, tryptamines given this way in cats anaesthetized with pentobarbitone failed to excite spinal neurones (Curtis & Davis, 1962), probably because pentobarbitone abolishes the stimulant actions of tryptamines on the spinal cord (Marley & Vane, 1963 ; Curtis, 1965). Such experiments should therefore be repeated without anaesthesia.

A depressant effect of indole compounds on the kneejerk, crossed extensor and ipsilateral flexor lower limb reflexes was observed by Marczyński (1962) in cats anaesthetized with urethane-chloralose. These indoles were substituted with heterocyclic

rings on the terminal nitrogen atom of the side-chain, a substitution which radically alters activity. This, together with his use of anaesthesia, makes comparison with our own results difficult.

The excitant activity of tryptamine derivatives on the spinal cord reinforces the possibility that one or more of them may have a physiological function as central nervous transmitters (see Douglas, 1965). Tryptamines also have other strong effects on the feline central nervous system, for example, on cortical neurones (Krnjević & Phillis, 1963) and on the lateral geniculate nucleus (Curtis & Davis, 1962). The possibility of transmitter function is strengthened by the presence of 5-hydroxytryptamine in the feline spinal cord (Anderson & Holgerson, 1966). Grey matter contained twice as much as white and the concentration of amine increased with descending segmental level. The amine was particularly localized to the areas in which motoneurones to the limbs originated, especially the lower limbs. Significant quantities were also found in the dorsal and ventral horns. The localization has been defined further by fluorescence microscopy. 5-hydroxytryptamine was confined to the terminal portion of axones descending from the brainstem and making contact with motoneurones in the anterior horn, with cells in the sympathetic lateral column and with neurones in the dorsal horn (Dahlström & Fuxe, 1965). These tryptaminergic neurones presumably activate motoneurones directly or indirectly by affecting internuncial neurones. The effects of injected tryptamines are most likely to be on the same receptor sites. However, the selective antagonism of the tryptamine effects by methysergide in doses which did not affect the flexor reflex suggests that tryptaminergic neurones are not directly involved in the polysynaptic reflex, but may only modulate the transmission in these pathways.

Although several metabolites were tested to see whether the after-depression of the flexor reflex induced by tryptamine could be explained by metabolic changes, none was found which caused the depression. Nevertheless, after a monoamine oxidase inhibitor given for sufficient time to ensure maximal potentiation of the substrate (Blackwell & Marley, 1966), the after-depression of the flexor reflex was not obtained with tryptamine. Thus, it may well be that an unidentified metabolite due to deamination of tryptamine is responsible for this depression. This possibility is rendered more likely by the absence of after-depression with $\alpha$-methyltryptamine which differs from tryptamine in that it is not easily deaminated.

The tryptamines altered the reflexes in an integrated fashion—that is, facilitated an ipsilateral flexor and corresponding contralateral extensor reflex. Tryptophan and 5-hydroxytryptophan, the immediate precursors of tryptamine and 5-hydroxytryptamine, given to cats pretreated with an amine oxidase inhibitor also had identical effects to the amines on the flexor reflex. The effects developed after a delay suggesting that the amines were being formed from the amino acids at sites within the spinal cord. A feature much more evident with the amino acids was the increased spinal cord excitability in the absence of increase in single twitch tension, as shown by enhanced responses to tetanizing the posterior tibial nerve.

Our results with tryptophan and 5-hydroxytryptophan agree well with those of Anderson & Shibuya (1966), except that they found depression, whereas we found potentiation, of the polysynaptic reflex after 5-hydroxytryptophan. If this difference were due

to the use of an amine oxidase inhibitor in our experiments, it would suggest that some metabolite of 5-hydroxytryptophan is normally formed which depresses the reflex.

SUMMARY

1. The effects of tryptamine were tested on flexor and extensor lower limb reflexes in spinal and decerebrate cats.

2. Tryptamine and $\alpha$-methyltryptamine increased basal tone and electromyographic activity in innervated but non-stimulated flexor (tibialis anterior) and contralateral extensor (quadriceps femoris) muscles.

3. Tryptamine and $\alpha$-methyltryptamine produced fusion or partial fusion of twitches evoked reflexly and increased peak and basal tensions in an ipsilateral flexor and a contralateral extensor reflex. Twitch duration was also increased and after $\alpha$-methyltryptamine oscillations appeared on the decaying part of the twitch, suggesting after-discharge. More prolonged effects were obtained with $\alpha$-methyltryptamine than with tryptamine.

4. Larger doses of tryptamine given intravenously or repeated small intra-aortic doses first enhanced, then reduced or suppressed twitch responses. The suppression did not appear to be due to the formation of tryptamine metabolites such as indol-3-ylacetic acid, 6-hydroxytryptamine, 5-methoxytryptamine or N-acetyl-5-hydroxytryptamine, but was not present in cats extensively pretreated with an amine oxidase inhibitor. Suppression was reversed by strychnine but not by $\alpha$-methyltryptamine nor by further doses of tryptamine.

5. Spinal cord potentials evoked by stimulating the divided dorsal root and recording from the divided corresponding ventral root were enhanced by tryptamine and $\alpha$-methyltryptamine. As with reflexes recorded myographically, the effects of $\alpha$-methyltryptamine were more prolonged; similarly, larger doses of tryptamine enhanced then diminished evoked potentials.

6. The effects of tryptamine on the flexor reflex were enhanced by pretreating cats with a hydrazide or a hydrazine monoamine oxidase inhibitor.

7. The effects of tryptamine on the flexor reflex were antagonized by methysergide; antagonism was surmountable. The effects were not antagonized by cocaine, phenoxybenzamine, or by chronic reserpinization.

8. Twitch tension was increased by L-tryptophan and DL-5-hydroxytryptophan in cats pretreated with an amine oxidase inhibitor. The effects of a tetanus to the central end of the divided posterior tibial nerve were also enhanced.

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