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Central Antiserotonin Action of Amitriptyline

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Summary

Amitriptyline (AMI) was studied in rats and mice in order to find out whether it had a central antiserotonin activity, previously demonstrated for doxepin – a compound chemically related to AMI. It was observed that AMI at low doses antagonized the head twitch response to L-5-hydroxytryptophan or 5-methoxytryptamine, as well as tryptamine-induced convulsions. In the hind limb flexor reflex preparation of the spinal rat AMI acted as a serotonin antagonist: when administered alone, it did not change the flexor reflex but prevented its stimulation induced by serotoninmimetics (LSD, quipazine, fenfluramine) not affecting that one evoked by noradrenalinemimetics (clonidine). At higher doses, AMI revealed a noradrenolytic activity. The results indicate that AMI, similarly as doxepin, has a central antiserotonin activity.

Zentrale antiserotonerge Wirkungen von Amitriptylin

Amitriptylin (AMI) wurde an Ratten und Mäusen in bezug auf zentrale antiserotonerge Wirkungen untersucht, die früher für Doxepin, ein Sauerstoffanalog von AMI, festgestellt wurden. Die Substanz antagonisiert in niedrigen Dosen den „head-twitch"-Effekt, der durch L-5-Hydroxytryptophan oder 5-Methoxytryptamin hervorgerufen wird. Die Tryptaminkrämpfe werden durch AMI auch blockiert. Auf den Flexor-Reflex der hinteren Pfote der spinalen Ratte wirkt AMI ähnlich wie bekannte Serotoninblocker; allein gegeben, läßt es ihn unbeeinflußt, antagonisiert aber den stimulierenden Effekt von serotonergen Substanzen (LSD, Quipazin, Fenfluramin). Die Ergebnisse weisen darauf hin, daß AMI – ähnlich wie Doxepin – eine zentrale antiserotonerge Wirkung besitzt.

Introduction

As we have found out recently, doxepin (DX) has a central antiserotonin activity (*Maj* et al. 1977b), similarly as cyproheptadine or metergoline, which are regarded as 5-hydroxytryptamine (5-HT) receptor blockers. Amitriptyline (AMI) is chemically related to DX, differing from the latter only by a CH_2 group in the central ring in place of an oxygen atom. Therefore, we decided to find out whether AMI also has a doxepin-like anti-5-HT action. For that purpose its effects on the head twitch response to L-5-hydroxytryptophan (L-5-HTP) or to 5-methoxytryptamine (5-MT), on tryptamine-induced convulsions, as well as on the hind limb flexor reflex of the spinal rat were investigated. The latter test appeared to be especially useful for assessing the 5-HT-agonistic or 5-HT-antagonistic activity (*Maj* et al. 1976, 1977c; *Palider* and *Rawtów* 1977).

Methods

Experiments were carried out on male Wistar rats and male Albino-Swiss mice, weighing 170–230 g and 20–25 g respectively. Food and water were available ad libitum prior to the experiments. AMI was administered in a 0.9% NaCl solution. The ED_{50s} of AMI with confidence limits were calculated according to *Litchfield* and *Wilcoxon* (1949).

Behavioural syndrome induced by L-5-HTP or by 5-MT. The influence of AMI on the L-5-HTP-induced syndrome was studied in rats and mice according to *Corne* et al. (1963). The ED_{50} values for AMI were assessed on the basis of the number of characteristic head twitches, counted at the following time intervals after L-5-HTP injection: 4–6, 14–16, 24–26, 34–36, 44–46, 54–56 min. The control value was 21 ± 3 in rats and 38 ± 5 in mice. AMI was administered i.p. 1 hr before L-5-HTP (270 mg/kg in rats, 280 mg/kg in mice, injected i.p. as a suspension in a 1% aqueous solution of Tween-80). Each dose was administered to 8 rats or 10 mice.

The effect on the head twitches induced by 5-MT in rats was investigated according to *Przegaliński* et al. (1977). The animals were given tranylcypromine (20 mg/kg i.p.) and, after 30 min, 5-MT (2,5 mg/kg i.p.), both dissolved in 0.9% NaCl. The head twitches were counted within a period of 15–60 min after 5-MT administration. Their number in control rats was 34 ± 6. AMI was injected i.p. 30 min before tranylcypromine. Each group consisted of 8 rats.

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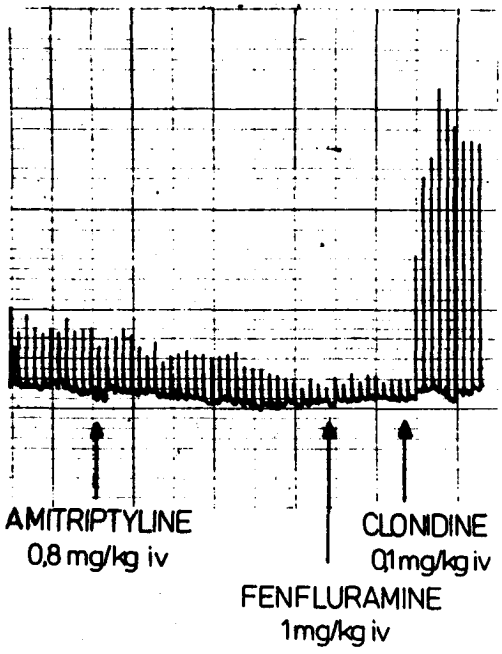

Fig. 1 The antagonistic influence of AMI on the effect of fenfluramine (1 mg/kg i.v.) and clonidine (0.1 mg/kg) on the hind limb flexor reflex of the spinal rat. AMI (0.8 mg/kg i.v.).was given 1 hr before fenfluramine. Clonidine was injected 20 min later.

The influence on the pinna reflex in rats was also tested according to *Corne* et al. (1963) at 15 min intervals, the measurements being started 1 hr after an i.p. injection of AMI. Each dose of AMI was given to 5 rats.

Convulsions induced by tryptamine. The rats were injected i.v. with tryptamine hydrochloride (40 mg/kg) dissolved in 0.9% NaCl. AMI was given i.p. 1 hr before. Each dose was given to 8 rats. Clonic convulsions of forepaws were assessed immediately after tryptamine injection, according to a conventional scale from 0 to 3 (1-single convulsions of forepaws; 2 – permanent convulsions of forepaws; 3 – permanent intensive forepaws and head convulsions). The value for control rats was 3.

Flexor reflex of the hind limb of the spinal rat. Experiments were carried out according to the technique described previously (*Maj* et al. 1976). The rat hind paw was stimulated through platinum electrodes inserted into the skin of the foot using electrical impulses (10–30 V, 10–50 msec at 1 min intervals). The contractions of the musculus tibialis anterior were recorded. All compounds were injected into the femoral vein. AMI was given 1 hr before the drugs used. Each dose was tested on 6–8 rats.

Drugs used: amitriptyline hydrochloride (Polfa), clonidine hydrochloride (Boehringer, Ingelheim), fenfluramine hydrochloride (Laboratories Servier), L-5-hydroxytryptophan (Sigma), delysid (LSD 25, Sandoz), 5-metoxytryptamine hydrochloride (Sigma), quipazine bimaleate (Miles), tranylcypromine sulfate (Smith, Kline and French), tryptamine hydrochloride (Polfa).

Results

Behavioural syndrome induced by L-5-HTP or 5-MT. The head twitch response to L-5-HTP is antagonized by AMI. Its ED_{50} values in rats and mice are 5.5 (2.29–13.2) mg/kg and 1.3 (0.76 2.21) mg/kg respectively.

AMI does not affect the pinna reflex in rats when administered in doses up to 40 mg/kg. Higher doses (60–80 mg/kg) abolish the righting reflex in part of the animals.

The head twitch response to 5-MT in rats is also blocked by AMI. Its ED_{50} is 17 (12.7–22.8) mg/kg.

Convulsions induced by tryptamine. AMI antagonizes convulsions induced by tryptamine in rats. Its ED_{50} value is 4.65 (2.65–8.13) mg/kg.

Flexor reflex of the hind limb of the spinal rat. AMI in doses of 0.8 and 1 mg/kg neither affects the flexor reflex of the spinal rat nor changes the stimulatory effect of clonidine (0.1 mg/kg). Doses of 4 mg/kg and higher depress the reflex or abolish it completely (8 mg/kg), also inhibiting the stimulatory effect of clonidine (0.1 and 0.2 mg/kg).

AMI in doses of 0.8–1.0 mg/kg counteracts the stimulation of the flexor reflex induced by fenfluramine (1 mg/kg), but not by clonidine (0.1 mg/kg) (Fig. 1); the stimulation caused by LSD (3 µg/kg) (Fig. 2) or quipazine (0.1 mg/kg) (Fig. 3) has also been reduced.

Discussion

The results presented here indicate that AMI antagonizes the head twitch response to L-5-HTP and 5-MT. The lack of effect on the pinna reflex proves that this antagonism does not result from a general depression of the central nervous system. The above findings, as well as the antagonism towards tryptamine-in-

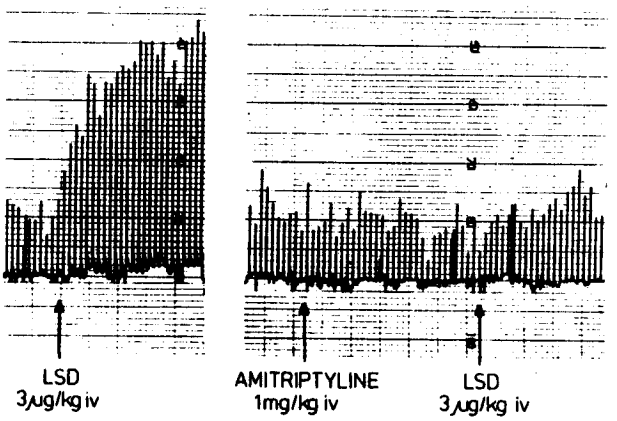

Fig. 2 The effect of AMI (1.0 mg/kg i.v.) on the LSD-induced (3 µg/kg i.v.) stimulation of the hind limb flexor reflex of the spinal rat. AMI was given 1 hr before the second injection of LSD.

duced convulsions, support the assumption that AMI reveals a central anti-5-HT activity.

The antagonism of AMI towards 5-HTP-induced head-twitches in mice was described by *Corne* et al. (1963), *Nakamura* et al. (1976); recently it was also demonstrated for AMI and nortriptyline (closely related to AMI) by *Fuxe* et al. (1977).

In the flexor reflex preparation of the spinal rat AMI, at doses which neither change this reflex nor affect its stimulation induced by a noradrenaline (NA) agonist (clonidine), inhibits the stimulatory action of 5-HT-mimetics – LSD and quipazine which act postsynaptically, and fenfluramine, revealing a presynaptic effect through 5-HT release. Therefore AMI behaves like the 5-HT antagonists previously studied by us, metergoline and cyproheptadine (*Maj* et al. 1976). At higher doses AMI has a noradrenolytic activity, which is indicated by the depression of the flexor reflex. The antagonism of AMI towards two other LSD-induced effects (head twitch response and extensor reflex stimulation) has been also observed (*Fuxe* et al. 1977).

The results presented point out that, in accordance with the assumption put forward in the Introduction, AMI reveals an activity similar to that of DX, a compound chemically related to it.

It is of interest that the binding studies point to a strong affinity of AMI to LSD-binding sites (antagonistic sites) of the 5-HT receptor

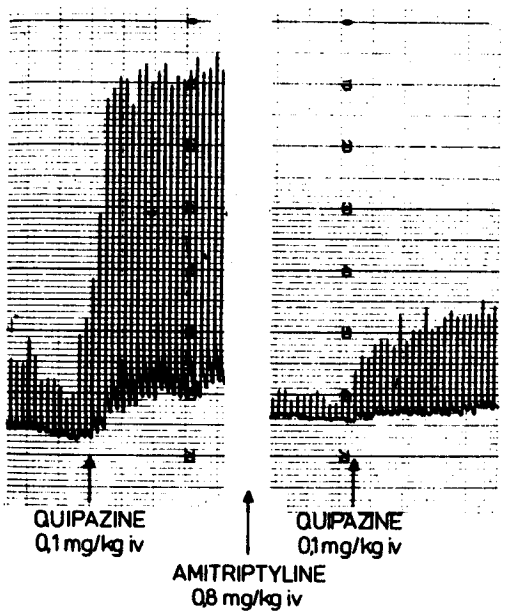

Fig. 3 The effect of AMI (90.8 mg/kg i.v.) on the quipazine (0.1 mg/kg i.v.)-induced stimulation of the hind limb flexor reflex of the spinal rat. AMI was given 1 hr before the second injection of quipazine.

in the rat cerebral cortex (*Bennet* and *Snyder* 1975; *Fuxe* et al. 1977); at the same time the experiments in rats with a lesion of the raphe nuclei indicate that the receptor in question is of a postsynaptic character (*Bennet* and *Snyder* 1975; *Fillion* et al. 1976; *Bennet* and *Aghajanian* 1974).

It is noteworthy that a distinct, peripheral anti-5-HT activity of AMI has been described previously (*Vernier* 1961, *Møller Nielsen* et al. 1966, *Buus Lassen* et al. 1975a).

Imipramine, and to a greater extent clomipramine and femoxetine which inhibit the 5-HT uptake, at doses of 1–10 mg/kg induce the flexor reflex stimulation, blocked by 5-HT antagonists (*Palider* and *Rawtów* 1977). AMI at any dose does not enhance the flexor reflex: conversely it inhibits – at a low dose (0.8 mg/kg) – its stimulation induced by 5-HT agonists.

Hence it may be assumed that the anti-5-HT activity of AMI is stronger than its inhibitory effect on the 5-HT uptake. It seems doubtful, therefore, whether inhibition of the 5-HT uptake induced by AMI may be relevant to its antidepressant action. At present a definite answer to this problem is impossible as, first of all, the above consideration concerns a single administration of AMI and the clinical antidepressant effect is obtained only after a longer period of treatment. It should be added here, that, in spite of the fact that AMI, being a tertiary amine, is commonly regarded as a drug strongly inhibiting the 5-HT uptake, some experimental data indicate that this effect is weaker than the inhibition of the NA uptake (*Buus Lassen* et al. 1975b), *Koe* 1976, *von Voigtlander* and *Losey* 1976), or does not occur in vivo at all (*Fuller* and *Wong* 1977, *Hyttel* 1977).

Our previous studies have demonstrated the central anti-5-HT-activity of other antidepressants: mianserin, trazodone, danitracen, DX (*Maj* et al. 1977 a, *Baran* et al. 1979, *Maj* et al. 1977 b). Recently *Fuxe* et al. (1977) have found the anti-5-HT activity for AMI and nortriptyline. DX and nortriptyline inhibit the NA uptake; hence this mechanism may account for their antidepressant activity. Mianserin, trazodone and danitracen have no effect on the NA uptake; therefore it is justifiable to consider their anti-5-HT activity to be the cause of the antidepressant effect. It may be assumed that the anti-5-HT activity disturbs the NA-5-HT interaction in favour of the former, and thus induces noradrenergic activation although via a different mechanism than the inhibition of the NA uptake.

There are a number of data supporting the existence of such an interaction (*Pujol* et al. 1978).

It should be added that electrophysiological data (*Itil* et al. 1972) point to the similarity of AMI to mianserin, a 5-HT antagonist. AMI, like mianserin, is also effective in the migraine treatment (*Couch* et al. 1976).

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