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Doxepin as a Blocker of Central Serotonin Receptors

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Summary

The antidepressant drug – Doxepin (DX) was examined in order to investigate its central antiserotonin activity. The drug antagonized the behavioral syndrome elicited by L-5-hydroxytryptophan in rats and mice, but did not affect the pinna reflex. In the flexor reflex preparation, DX acted like other serotonin receptor blockers: By itself, it had no influence on the flexor reflex but it prevented the potentiation induced by serotonergic agents (fenfluramine, LSD, mescaline). The hyperthermia provoked by serotonergic agents (fenfluramine, LSD) in rabbits was antagonized by DX. DX abolished the syndrome induced by oxotremorine. The results obtained indicate that DX blocks central 5-HT receptors, like the two other antidepressants, mianserin and danitracen.

Zusammenfassung

Doxepin (DX), ein Antidepressivum, wurde in bezug auf zentrale antiserotonerge Wirkungen an Ratten, Mäusen und Kaninchen untersucht. Die Substanz antagonisiert in niedrigen Dosen das Verhaltens-Syndrom, welches durch L-5-Hydroxytryptophan hervorgerufen wird, ohne den Pinna-Reflex zu beeinflussen. Auf den Flexor-Reflex der hinteren Pfote der spinalen Ratte wirkt DX ähnlich wie Serotonin-Blocker – allein gegeben ist es ohne Einfluß, antagonisiert aber den stimulierenden Effekt von serotonergen Substanzen (Fenfluramin, LSD, Meskalin). Die durch Fenfluramin oder LSD hervorgerufene Hyperthermie an Kaninchen wird durch DX blockiert. Die Ergebnisse beweisen, daß DX – ähnlich wie zwei andere Antidepressiva, Mianserin und Danitracen – eine zentrale antiserotonerge Wirkung besitzt.

Introduction

Mianserin and Danitracen are clinically active antidepressants (*Itil* et al., 1972; *Fell* et al., 1973; *Matussek* et al., 1976) although they do not induce the pharmacological effects regarded as characteristic for this group of drugs (*van Riezen,* 1972; *Kafoe* and *Leonard,* 1973; *Leonard,* 1974; *Kähling* et al., 1975; *Maj* et al., 1976a, 1977a). Both drugs possess distinct peripheral and central antiserotonin activities (*Vargaftig,* 1971; *van Riezen,* 1972; *Engelhard,* 1975; *Maj* et al., 1976a, 1977a).

Published results (see *Pinder* et al., 1977) show that doxepin (DX), another clinically active antidepressant, displays a weak tricyclic antidepressant (TAD) action in animals. The antagonistic action of DX against reserpine and potentiation of an amphetamine effect are slight or even absent. Its inhibition of noradrenaline (NA) and 5-hydroxytryptamine (5-HT) uptake in animals and patients is also moderate, as compared with other TADs (see *Pinder* et al., 1977). For instance, according to *Buus Lassen* (1975a), DX was about 10 and 50-100 times less potent than imipramine and clomipramine respectively in blocking brain 5-HT uptake and about 2-3 and 100 times less potent than imipramine and clomipramine respectively in blocking brain NA uptake.

For the above reasons, we decided to investigate whether DX possesses central antiserotonin activity. A survey of the literature has revealed no results concerning this questin (see Discussion). In the present work, three tests were used: "behavioral" syndrome elicited by L-5-hydroxytryptamine (5-HTP), the flexor reflex of the hind limb of the spinal rat, and body temperature in rabbits.

5-HTP induces a characteristic syndrome which is antagonized by low doses of 5-HT blockers (*Corne* et al., 1963; *Jacobs,* 1976). This antagonistic effect is considered the more specific, the greater the difference is between doses of 5-HT blockers active in this test and those inhibiting the pinna reflex (*Corne* et al., 1963).

The flexor reflex of the spinal rat proved to be a suitable preparation to asses central serotonergic and antiserotonergic activity (see Discussion). Body temperature in rabbits is increased by 5-HT stimulants but this hyper-

thermic effect is prevented by 5-HT receptor blockers (see Discussion). Central cholinolytic activity of DX was also assessed, based on its antagonistic action to oxotremorine. In some experiments, appropriate substances were used for comparison.

Methods

Experiments were carried out on animals having free access to food and water; male Wistar rats, male Albino-Swiss mice and male White-Danish rabbits weighing 130-180 g, 18-22 g and 1.8-3 kg respectively. All compounds were administered in 0.9 % NaCl solution. Animals in control groups were given corresponding volumes of the solvent.

Behavioral syndrome induced by 5-HTP. Experiments were performed according to *Corne* et al. (1963). One hour after DX, imipramine or atropine administration, rats and mice were injected with 5-HTP (as a suspension in 1 % aqueous solution of Tween 80) in doses of 270 and 280 mg/kg respectively. All drugs were given intraperitoneally. Characteristic "head twitches" were counted 6 times at the following intervals after administration of 5-HTP: 4-6, 14-16, 24-26, 34-36, 44-46, 54-56 minutes. Each group comprised 6-8 rats or 8-10 mice. ED_{50} was calculated according to *Litchfield* and *Wilcoxon* (1949).

The influence on the pinna reflex was tested in rats and mice according to *Corne* et al. (1963) by irritating the external auditory meatus with a fine hair at 60, 75, 90, 105 and 120 minutes after i.p. injection of DX or imipramine.

The flexor reflex of the hind limb of the spinal rat. Experiments were carried out according to the technique described earlier (*Maj* et al., 1976b). The rat hind paw was stimulated electrically and contractions of the musculus tibialis anterior were recorded. All compounds were administered into the femoral vein, and DX was given 1 hour before the agonists.

Body temperature in rabbits. Body temperature was measured rectally by means of an Ellab thermometer. Immobilized animals were adapted to the ambient temperature of 22 ± 1° C for 2 hours. Two initial temperatures were taken at an interval of 30 minutes, then DX was administered and the body temperature was measured at 30 minute intervals. Fenfluramine and LSD were given 1 hour after injection of DX. All drugs were administered into the marginal ear vein. Results were presented as mean ± SEM. Statistical significance was calculated using the Student's t-test.

Oxotremorine syndrome. Experiments were carried out on mice injected i.p. with oxotremorine in a dose of 1 mg/kg. DX was given i.p. 1 hr before. Tremor, salivation and lacrimation were assessed by an arbitrary scale ranging from 0 to 3 scores (0 – no symptoms present, 3 – the highest intensity of symptoms). The ED_{50} was calculated using the method of *Litchfield* and *Wilcoxon* (1949) the effect being observed 30 minutes after oxotremorine administration.

Drugs used: atropine sulfate (Polfa), doxepin hydrochloride (DX, Pfizer), fenfluramine hydrochloride (Laboratoires Servier), L-5-hydrocytryptophane (5-HTP, Sigma), imipramine hydrochloride (Polfa), lysergamid (LSD, Spofa). mescaline sulfate (Fluka), oxotremorine oxalate.

Results

Behavioral syndrome induced by 5-HTP. DX antagonized the 5-HTP-induced syndrome. The ED_{50} for rats and mice was 4.43 (1.65-11.9) mg/kg and 3.22 (1.51-6.85) mg/kg respectively. The 5-HTP effect was also blocked by imipramine, the ED_{50} being for rats 16 (9.3-27.7) mg/kg and for mice 12.8 (7.3-22.4) mg/kg. Atropine (2.5, 5 and 10 mg/kg) did not affect the 5-HTP-induced-syndrome. DX in doses of 5, 10, 25, and 50 mg/kg had no influence on the pinna reflex in rats and mice. A dose of 100 mg/kg abolished the righting reflex and was lethal for some of the animals.

Imipramine in doses upt to 40 mg/kg did not change the pinna reflex in rats. In mice, the inhibiting effect was observed, the ED_{50} being about 5 mg/kg.

The flexor reflex of the hind limb of the spinal rat. DY in a dose of 2 mg/kg i.v. did not affect the flexor reflex of the spinal rat but blocked the stimulation induced by fenfluramine (1 mg/kg i.v.), LSD (9 mg/kg i.v.) or mescaline (3 mg/kg i.v.) (Fig. 1); fenfluramine-induced stimulation has not been shown in the figure but it was always seen after the dose used (*Maj* et al., 1976b and 1977c). The lowest effective dose of DX was 0.5-1 mg/kg i.v.

At higher doses (3-10 mg/kg), DX given alone still did not influence the flexor reflex. In some experiments, however, after a dose of 10 mg/kg, a depression of the flexor reflex was observed (Fig. 2).

Body temperature in rabbits. Fenfluramine (10 mg/kg i.v.) induced hyperthermia a reaction which was inhibited by DX in a dose of 3 mg/kg i.v. (Fig. 3). Lower doses of DX had

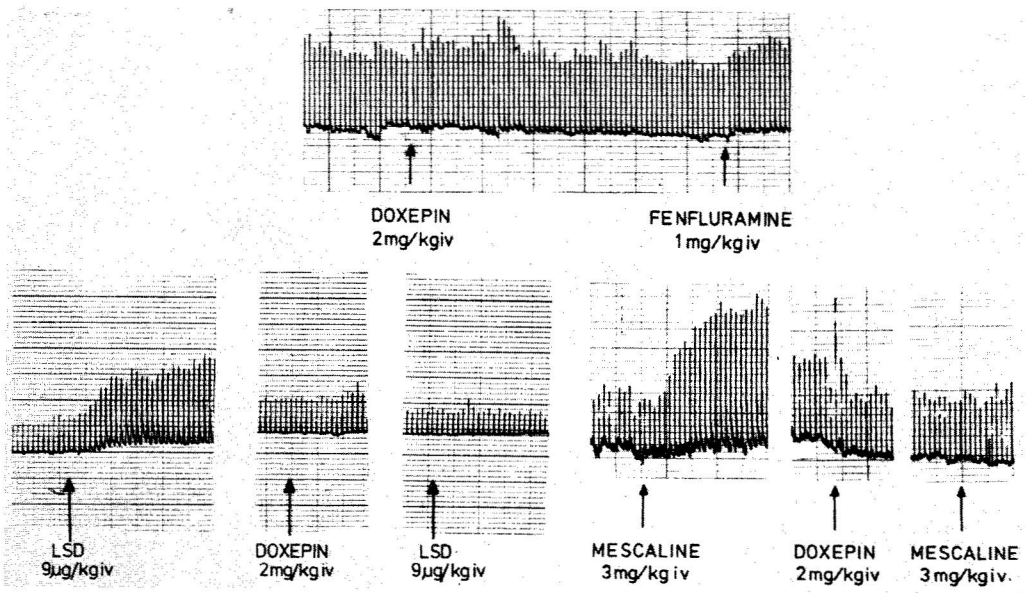

Fig. 1: The effect of fenfluramine (1 mg/kg i.v.), LSD (9µg/kg i.v.) and mescaline (2 mg/kg i.v.) on the flexor reflex of the hind limb of the spinal rat after pretreatment with DX (2 mg/kg i.v.).

a weak, statistically insignificant antagonistic effect. DX given alone induced a fall in body temperature of 1.3° C.

LSD in a dose of 150 µg/kg caused a hyperthermic effect of 2° C, maximally (Fig. 4); this effect was blocked by DX in a dose of 6 mg/kg. DX given alone decreased body temperature by about 0.7° C.

Oxotremorine syndrome. DX in doses of 3, 6, 12 and 24 mg/kg i.p. abolished tremor, lacrimation and salivation produced by oxotremorine (1 mg/kg i.p.) in mice. The most pronounced antagonism, appearing after a dose of 3 mg/kg, was observed against tremor. The ED_{50} of DX inhibiting the intensity of tremor, lacrimation and salivation was 5 (3.79-6.6) mg/kg, 10.9 (10.14-11.72) mg/kg and 12.5 (9.06-17.2) mg/kg for each symptom respectively.

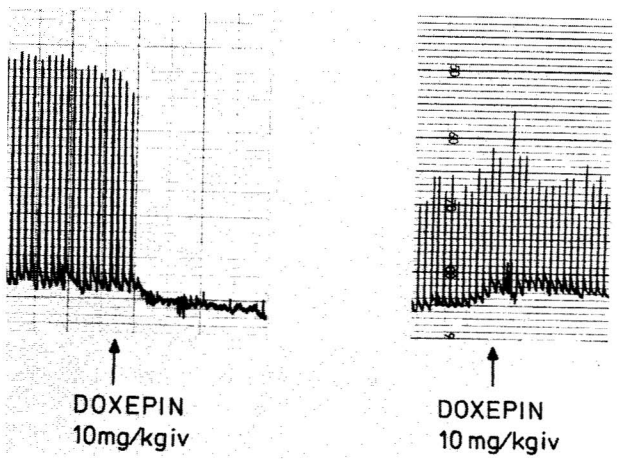

Fig. 2: The influence of DX (10 mg/kg i.v.) on the flexor reflex of the hind limb of the spinal rat.

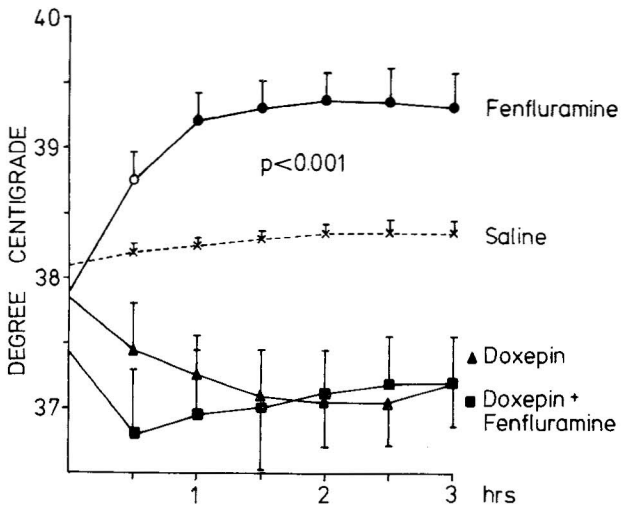

Fig. 3: The influence of DX (3 mg/kg i.v.) on the hyperthermic effect of fenfluramine (10 mg/kg i.v.) in rabbits. The results are presented as means ± SEM. The statistical significance of the results was controlled by the Student t-test. Solid symbols indicate the statistically significant results.
0——0 vs X---X, $p < 0.01$;
Δ——Δ vs X---X, $p < 0.01$;
□——□ vs 0——0, $p < 0.001$.

Discussion

In order to investigate the central antiserotonin activity of DX, three tests were used and three species of animals subjected to experiments. In the first test, the behavioral syndrome elicited by 5-HTP was inhibited, both in rats and mice, by DX. Lack of influence on the pinny reflex indicates that this antagonism to the 5-HTP syndrome does not result from general depression of the central nervous system. Furthermore, doses of DX effective in this test are lower than those active on the central nervous system as estimated in a variety of other tests (see *Pinder* et al., 1977). One may, thus, suppose that the antagonism of DX to 5-HTP is caused by antiserotonin activity.

Also, imipramine abolishes the 5-HTP-induced syndrome in rats and mice. Similar results were obtained in mice by *Corne* et al. (1963), though the ED_{50} found by these authors was fivefold higher than that calculated from our experiments. The mechanism of this antagonistic action of imipramine is obscure. Its influence on the pinna reflex in mice indicates an unspecific depressive activity. *Przegaliński* et al.

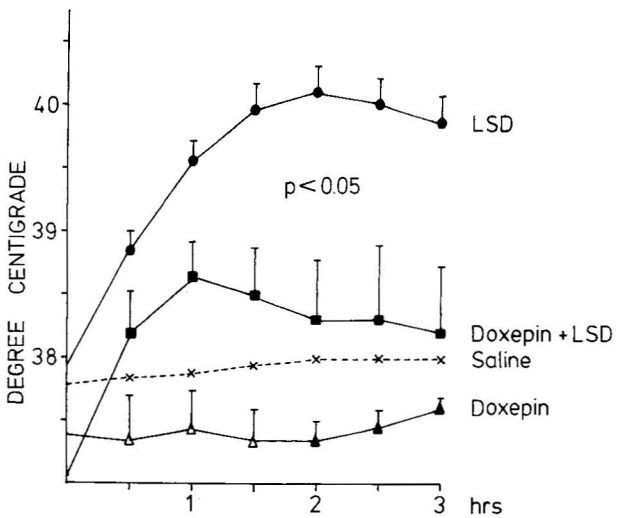

Fig. 4: The influence of DX (6 mg/kg i.v.) on the hyperthermic effect of LSD (150 μg/kg i.v.) in rabbits. The results are presented as means ± SEM. The statistical significance of the results was controlled by the Student t-test. Solid symbols indicate the statistically significant results.
0——0 vs X---X, $p < 0.001$;
Δ——Δ vs X---X, $p < 0.05$;
□——□ vs 0---0, $p < 0{,}05$.

(1977) have also described small differences between doses of imipramine inhibiting head twitches elicited by 5-methocytryptamine and those abolishing the pinna reflex. It seems improbable that the antagonistic effect of imipramine against 5-HTP can be attributed to its main mechanism i.e. inhibition of monoamine uptake. If this were the case, the effective doses of imipramine would be lower than those of DX, for the latter is a much weaker antidepressant. On comparing the ED_{50} obtained in our experiments on rats and mice in the 5-HTP-test, DX appears to be about fourfold more potent than imipramine.

The results of the oxotremorine test indicate that DX possesses cholinolytic activity in doses similar to those effective in the 5-HTP test. Since atropine is inactive in this test it can be assumed that the cholinolytic activity of DX is unrelated to its anti-5-HTP effect.

As was demonstrated in our previous experiments, fenfluramine, LSD, mescaline, tryptophan, 5-HTP, chloramphetamine and quipazine all potentiate the flexor reflex of the spinal rat. This potentiation is blocked by pretreatment with 5-HT receptor blockers: cyproheptadine, mianserin, danitracen and methergoline although these drugs by themselves, have no influence on the flexor reflex (*Maj* et al., 1976b, 1977b, c; *Palider* and *Rawłów,* 1977). As may be concluded from the results presented here, DX resembles a 5-HT blocker in that it itself has no influence on the flexor reflex but prevents the potentiation caused by fenfluramine, LSD and mescaline. High doses of DX are able to depress the flexor reflex. This finding indicates that DX in higher doses may be a blocker of central NA receptors.

The hyperthermic response to serotonergic agents e.g. LSD or fenfluramine in rabbits is antagonized by 5-HT antagonists such as cyproheptadine (*Horita* and *Hill,* 1972; *Quock* and *Beal,* 1976), danitracen and mianserin (*Maj* et al., 1977a). Thus, by counteracting the hyperthermic effect of LSD and fenfluramine, DX acts as a 5-HT receptor blocker. One cannot exclude the possibility that the antagonism of DX against fenfluramine results, in part, from its presynaptic action inhibiting the uptake of fenfluramine by the 5-HT neuron. A similar action of imipramine has been described (*Christopher* and *Sally,* 1974). The antagonism against fenfluramine in the flexor reflex preparation has also been found in our previous experiments (*Palider* and *Rawłów,* 1977).

Thus, the results obtained in these three tests indicate a central 5-HT receptor blocking activity of DX. Some other observations would substantiate such an action. Antiserotonin activity of DX was demonstrated on the isolated ileum and trachea of the guinea pig being in the former preparation, only 6 times weaker than cyproheptadien (*Constantine* et al., 1964). The hypotensive effect of 5-HT in rats in antagonized by DX (unpublished data). DX (*d'Elia* et al., 1974; *Forsen,* 1975), like cyproheptadine (*Silverstone* and *Schuyler,* 1975), increases body weight in patients. The latter drug, as well as danitracen, (*Kähling* et al., 1975) increases food intake in animals and this effect probably results from blockade of the central 5-HT receptors.

Thus, besides mianserin and danitracen, DX also seems to be an antidepressant drug possessing antiserotonin activity. Other experiments (*Maj* et al., 1976a, 1977a) have shown that this activity of DX, especially in the 5-HTP syndrome test, is weaker than that of mianserin or of danitracen.

The finding of 5-HT receptor blocking activity does not mean, of course, that DX does not possess a presynaptic action inhibiting 5-HT uptake. This effect, as demonstrated in a variety of experiments (e.g. brain structures, human and animal blood platelets), is weak compared with that of TAD (see *Pinder,* 1977; *Buus Lassen* et al., 1975a, b). Since the inhibition of monoamine uptake and blockade of postsynpatic receptors results in a functionally opposite effect, the question as to which effect predominates is of vital importance. The results presented here indicate that the postsynaptic receptor blockade effect to prevails. This theory is supported by a comparison of the doses effective in our experiments on the rat (2-4 mg/kg) with a dose giving 50 % reversal of p-chloramphetamine-induced 5-HT depletion from rat brain (13 mg/kg) (*Buus Lassen* et al., 1975a).

It is interesting that DX, mianserin and danitracen have, apart from the antiserotonin activity, still another common property, i.e. antihistaminic activity. Mianserin and danitracen are even more potent antihistaminics. DX, on the preparation of the isolated ileum of the guinea pig, acts 100 times and 10 times stronger than tripelenamine (*Constantine* et al., 1964) and diphenhydramine (*Buus Lassen* et al., 1975a) respectively. Whether this antihistaminic activity, demonstrated on a peripheral organ, is associated with the central antiserotonin or antidepressant action is unknown.

Cholinolytic activity is a characteristic of many antidepressants. As indicated by literature data (see *Pinder,* 1977) and by our results of the oxotremorine test, DX pssesses such activity, as well as danitracen (*Engelhard,* 1975; *Kähling* et al., 1975; *Maj* et al., 1976a) but not mianserin (*Vargaftig* et al., 1971; *van Riezen,* 1972; *Maj* et al., 1977a).

The mechanism of the antidepressive action of mianserin and danitracen, 5-HT receptor blockers, still remains obscure. Also, one cannot answer the question as to whether the 5-HT receptor blocking activity of DX is of importance for its antidepressive efficacy. However, this does not alter the fact that DX, as shown in our experiments, does exhibit such central anti-5-HT activity.

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