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Effect of butriptyline on the brain uptake mechanisms for noradrenaline and 5-hydroxytryptamine

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Butriptyline, a tricyclic compound (40 mg kg^{-1}, i.p.), in contrast to the tricyclic desipramine (20 mg kg^{-1}, i.p.), did not alter the accumulation or metabolism in areas of the rat brain of intraventricularly-injected [^{3}H]noradrenaline. It did not appreciably inhibit the displacement of 5-HT by α-ethyl-3-hydroxy-4-methyl phenethylamine in comparison with the tricyclic chlorimipramine. Butriptyline potentiated central 5-HTP effects only slightly whereas chlorimipramine displayed a strong potentiation. It is concluded that butriptyline differs significantly from desipramine, as no inhibitory effect on brain noradrenaline uptake was found, and from chlorimipramine, as only a weak inhibitory activity on brain 5-HT uptake was observed.

Butriptyline * is a tricyclic compound possessing a neuropsychopharmacological profile in animals similar to that of various tricyclic antidepressants (Voith & Herr, 1969; Herr, Voith & Jaramillo, 1971) and clinically is an antidepressant agent (González & Montaño, 1971; Fiume, 1971; Grivois, 1971; Alves-Garcia, 1971; Perez de Francisco, Nieto Gomez & others, 1971). The drug has been shown to be devoid of a blocking effect on noradrenaline uptake in mouse and rat heart (Lippmann, 1969, 1971). In the present studies its effects on the noradrenaline and 5-hydroxytryptamine and (5-HT) uptake mechanisms in the brain have been assessed.

METHODS AND MATERIALS

Injection of radioactive materials and dissection procedure

Sprague-Dawley male rats (150–160 g, Canadian Breeding Laboratories) were treated with butriptyline (40 mg kg^{-1}, i.p.), desipramine (20 mg kg^{-1}, i.p.) or saline and 1 h later they were lightly anaesthetized with ether and (±)-7[^{3}H]noradrenaline (7 μCi in 20 μl saline per rat) was injected into the lateral ventricle of the brain according to Noble, Wurtman & Axelrod (1967). One h later the rats were decapitated, the brains removed, rinsed in ice-cold saline, the hypothalamus and medulla (medulla oblongata and pons) were dissected out as described by Glowinski & Iversen (1966).

Isolation and assay of catecholamines and their metabolites

Tissues were weighed and homogenized in 5 ml of cold 0·4N perchloric acid containing 0·1% EDTA and 0·1% ascorbic acid. After centrifugation, total radioactivity,

* Evadyne, Ayerst Laboratories.

([^{3}H]noradrenaline (^{3}H-NA) and its deaminated metabolites [^{3}H]3,4-dihydroxy-mandelic acid and [^{3}H]3,4-dihydroxy-phenylglycol), was measured by the alumina absorption method of Whitby, Axelrod & Weil-Malherbe (1961). The [^{3}H] metabolites were extracted from alumina eluates into ethyl acetate at pH 1 (Kopin, Axelrod & Gordon, 1961) and the content of ^{3}H-NA found by difference. The *O*-methylated metabolite of noradrenaline, [^{3}H]normetanephrine was isolated from the alumina column effluents on a Dowex 50W-X-8 column (200–400 mesh, NH_4^+-form), and eluted with 3N NH_4OH (Kopin & others, 1961). The free *O*-methylated, deaminated metabolites of noradrenaline (i.e. [^{3}H]vanillyl-mandelic acid and [^{3}H]4-hydroxy-3-methoxy-phenylglycol), were estimated by the difference between total radioactivity of tissue extracts and the sum of ^{3}H-NA and the other metabolites. Radioactive standards were carried through the extraction procedure. The recovery of added standard ^{3}H-NA and [^{3}H]normetanephrine averaged 74 $\pm$ 1% and 61 $\pm$ 2% (mean $\pm$ s.e., n = 10), respectively. All results were corrected for recovery. The radioactivity in sample aliquots was determined with 10 ml of Aquasol scintillation fluid and liquid scintillation counting. The counting efficiency for ^{3}H was 27%.

Potentiation of the pharmacological effects of 5-hydroxytryptophan (5-HTP)

The method of Carlsson, Jonason & others (1969b) was used. Male albino mice (23–25 g; Canadian Breeding Laboratories) were given drugs 30 min before 5-HTP (300 mg kg^{-1}, i.p.). The strength of the 5-HTP behavioural syndrome characterized by tremor, lordosis, abduction of the hind-legs and head twitches is indicated by an arbitrary scale ranging from 0 (no effect) to +4 (very strong effect), 15 min after injection of 5-HTP.

Displacement of 5-HT in rat brain by α-ethyl-3-hydroxy-4-methyl-phenethylamine HCl (EMT)

Male Sprague-Dawley rats (140–160 g, Canadian Breeding Laboratories) were injected with EMT in two doses of 25 mg kg^{-1}, i.p., 2 h apart and the animals killed 2 h later. The brains were removed, rinsed in cold saline, blotted, weighed and frozen over dry ice for assay. Butriptyline and chlorimipramine were injected intraperitoneally, 30 min before each dose of EMT, the second dose of the drugs being half the first.

Whole brain 5-HT was extracted by the method of Maickel, Cox & others (1968) and assayed fluorometrically (Bogdanski, Pletscher & others, 1956). The recovery of 5-HT added to the brain before homogenization averaged 65 $\pm$ 1% (mean $\pm$ s.e., n = 5). All results were corrected for recovery.

The percentage inhibition by the drugs of the 5-HT displacement by EMT was calculated from the formula of Bruinvels (1971).

Desipramine hydrochloride (Pertofran) and chlorimipramine hydrochloride (Anafranil) were kindly donated by Ciba–Geigy Ltd. α-Ethyl-3-hydroxy-4-methyl-phenethylamine hydrochloride (EMT), (±)-[7-H^3]noradrenaline hydrochloride (specific activity 6·48 Ci mmol) and DL-5-hydroxytryptophan monohydrate were purchased from Aldrich Chemical Co., New England Nuclear Corp., and Calbiochem, respectively.

RESULTS

Effects on the accumulation and metabolism of ^{3}H-NA

Butriptyline did not inhibit the accumulation of ^{3}H-NA in either the hypothalamus (Table 1A) or medulla (Table 1B). In contrast, desipramine, a known blocker of noradrenaline uptake in the brain (Glowinski, Axelrod & Iversen, 1966), significantly inhibited ^{3}H-NA accumulation in both areas, the effect in the medulla being the greater.

Desipramine altered the pattern of ^{3}H-NA metabolites in both brain areas (Table 1) while butriptyline had no significant effect on ^{3}H-NA metabolism.

Table 1. *Effects on accumulation and metabolism of ^{3}H-NA in the medulla (medulla oblongata and pons) and hypothalamus of rats.* Rats were administered butriptyline (40 mg kg^{-1}, i.p.), desipramine (20 mg kg^{-1}, i.p.) or saline 1 h before an intraventricular injection of ^{3}H-NA and were killed 1 h after the latter treatment. Values are expressed as % ± s.e. of control levels for ^{3}H-NA and each of the metabolites. Each value is the mean of 9–10 animals.

Treatment	^{3}H-NA	^{3}H-DH (% of control ± s.e.)	^{3}H-OM	^{3}H-OMDH
A. Hypothalamus				
Saline	100·0 ± 5·9	100·0 ± 5·7	100·0 ± 4·5	100·0 ± 10·1
(Counts × 10^4 min^{-1}g^{-1})	(68·99 ± 4·09)	(3·84 ± 0·22)	(10·12 ± 0·45)	(55·7 ± 5·64)
Butriptyline	102·5 ± 5·9	118·8 ± 6·5*	106·2 ± 5·3	99·3 ± 5·0
Desipramine	83·2 ± 2·7**	69·3 ± 3·8***	147·2 ± 5·9***	125·1 ± 4·5*
B. Medulla				
Saline	100·0 ± 7·0	100·0 ± 3·9	100·0 ± 7·6	100·0 ± 7·8
(Counts × 10^4 min^{-1}g^{-1})	(31·36 ± 2·20)	(3·47 ± 0·14)	(9·28 ± 0·70)	(32·70 ± 2·54)
Butriptyline	112·3 ± 10·9	109·2 ± 11·0	114·9 ± 10·4	121·1 ± 11·9
Desipramine	68·2 ± 3·8***	38·3 ± 1·8***	175·3 ± 14·6***	126·3 ± 5·8**

*** P <0·001; ** <0·02; * 0·05 when compared with saline.

^{3}HDH = dihydroxymandelic acid and dihydroxyphenylglycol. ^{3}H-OM = normetanephrine. ^{3}H-OMDH = vanillylmandelic acid and hydroxymethoxyphenylglycol.

Table 2. *Effect of butriptyline and chlorimipramine on the displacement of brain 5-HT by EMT in rats.* Rats were injected with α-ethyl-3-hydroxy-4-methyl-phenethylamine (EMT) in two doses of 25 mg kg^{-1}, i.p., 2 h apart and the animals killed 2 h later. Drugs were administered 30 min before each dose of EMT, the second dose of the test drug being half the first. The figures in parentheses represent the number of animals per group.

Treatment	First dose (mg kg^{-1}, i.p.)	Brain 5-HT (μg g^{-1} ± s.e.) Drug alone	Drug + EMT	Inhibition (%)	ED50
Saline	—	0·49 ± 0·02 (8)	0·28 ± 0·01 (8)		
Butriptyline	50	0·49 ± 0·02 (5)	0·33 ± 0·02 (5)*	23	>50
	25	0·47 ± 0·02 (5)	0·30 ± 0·01 (5)		
	12·5	0·43 ± 0·01 (5)	0·29 ± 0·01 (5)		
Chlorimipramine	25	0·46 ± 0·01 (5)	0·40 ± 0·01 (5)***	70	12·5
	12·5	0·46 ± 0·03 (5)	0·36 ± 0·02 (5)**	49	
	6·25	0·45 ± 0·03 (5)	0·32 ± 0·01 (5) *	33	

*** P <0·001; ** <0·01; * <0·05 as compared to saline + EMT group.

Effects on displacement of 5-HT by EMT

Rats administered EMT exhibited decreased levels of 5-HT in the brain (Table 2). Butriptyline prevented this decrease to a slight degree but only at the highest dose examined (ED50 >50 mg kg^{-1}, i.p.). In contrast, chlorimipramine, a known blocker of 5-HT uptake (Carlsson, Corrodi & others, 1969a) inhibited the EMT-induced depletion of 5-HT at all doses examined (ED50: 12·5 mg kg^{-1}, i.p.). Neither drug alone affected 5-HT levels.

Potentiation of the pharmacological effects of 5-HTP

Butriptyline potentiated the 5-HTP behavioural response but only at a relatively high dose (i.e. 50 mg kg^{-1}, i.p.) giving a score of 2 while chlorimipramine, a known potentiator (Carlsson & others, 1969b), potentiated the 5-HTP syndrome at 25 and 12·5 mg kg^{-1} (i.p.) with scores of 4 and 3, respectively.

DISCUSSION

The results indicate that butriptyline, unlike desipramine, did not inhibit the brain noradrenaline re-uptake mechanism *in vivo*. In addition, butriptyline, in contrast to chlorimipramine, exhibited only weak activity in blocking the active transport mechanism for brain 5-HT.

The effect of the drugs on brain noradrenaline uptake and metabolism were studied by employing intraventricularly injected ^{3}H-NA. With this technique, it has been shown that tricyclic antidepressants, e.g. desipramine, inhibit ^{3}H-NA accumulation in the hypothalamus, medulla oblongata, and cerebellum with only small changes in other areas (Glowinski & others, 1966) and also alter levels of ^{3}H-NA metabolites (Glowinski & Axelrod, 1966). The findings for desipramine were confirmed which is consistent with an action of blockade of the noradrenaline membrane pump. Butriptyline, however, affected neither the accumulation of ^{3}H-NA nor its subsequent metabolism. This lack of activity reflects previous findings for butriptyline where no effect on noradrenaline uptake was observed in either rat or mouse heart (Lippmann, 1969, 1971).

Prior treatment with tricyclic antidepressants, e.g. chlorimipramine, imipramine and amitriptyline has been shown to block the displacement of 5-HT by EMT, an α-methyltyramine-type compound; this has been interpreted as being due to blockade of the 5-HT membrane pump by which EMT gains access to the 5-HT stores (Carlsson & others, 1969a). Butriptyline exerted only slight activity in preventing the EMT-induced depletion of brain 5-HT compared to chlorimipramine indicating an ineffective blockade of the 5-HT membrane pump.

Potentiation of the effects of a subthreshold dose of 5-HTP by tricyclic antidepressants is also considered to reflect a blockade of the 5-HT membrane pump (Carlsson & others, 1969b). The weak potentiation of 5-HTP by butriptyline, in contrast to the strong potentiation by chlorimipramine, is consistent with the biochemical findings in this respect.

Thus, while some tricyclic compounds used to treat depression have previously been shown by biochemical studies to inhibit the noradrenaline or 5-HT neuronal membrane pumps, or both, butriptyline, also a tricyclic antidepressant, differs significantly from desipramine in having no effect on brain noradrenaline uptake, and from chlorimipramine, in having only a slight effect on brain 5-HT uptake.

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