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EFFECTS OF BENZOCTAMINE AND CHLORDIAZEPOXIDE ON TURNOVER AND UPTAKE OF 5-HYDROXYTRYPTAMINE IN THE BRAIN

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1 Benzoctamine, a new psychoactive drug, known to exert in man an anti-anxiety effect resembling that of chlordiazepoxide, decreased the disappearance of intraventricularly-injected [^{14}C]-5-hydroxytryptamine (5-HT) from rat brain, as did chlordiazepoxide.

2 Both drugs partially inhibited the α-ethyl-3-hydroxy-4-methylphenylethylamine-induced depletion of rat brain 5-HT.

3 It is concluded that benzoctamine, like chlordiazepoxide, decreases 5-HT turnover in the brain and that this action may play a role in the anti-anxiety effect of these drugs observed in man.

Introduction

Studies from several laboratories have demonstrated that minor tranquillizers such as benzodiazepines (Taylor & Laverty, 1969; Chase, Katz & Kopin, 1970; Corrodi, Fuxe, Lidbrink & Olson, 1971; Wise, Berger & Stein, 1972), and meprobamate (Lidbrink, Corrodi, Fuxe & Olson, 1972) decrease the rate of turnover of brain noradrenaline (NA), dopamine (DA) and/or 5-hydroxytryptamine (5-HT). The alterations in monoamine turnover are thought to be at least partly responsible for some of the pharmacological and behavioural effects observed following treatment with these drugs.

Benzoctamine, a new psychoactive drug, exerts an anti-anxiety effect in humans (Goldstein & Weiner, 1970; Forrest, 1972). Its spectrum of activity, in both pharmacological and biochemical studies, is distinct from other psychoactive drugs such as imipramine, chlorpromazine, reserpine and diazepam (Bein, 1970; Maître, Staehelin & Bein, 1970).

Recently, it has been suggested that at least part of the anti-anxiety effect of the benzodiazepines is due to decreased activity of 5-HT neurones (Wise *et al.*, 1972). In the present study effects of benzoctamine on 5-HT metabolism have been determined and a comparison made with the effects of chlordiazepoxide.

Methods

Groups of rats (male albino 140-150 g; Sprague Dawley, Canadian Breeding Laboratories) received [^{14}C]-5-HT (0.35 μc) into the lateral ventricle (Noble, Wurtman & Axelrod, 1967) 10 min prior to the administration of benzoctamine (20 mg/kg, i.p.), chlordiazepoxide (20 mg/kg, i.p) or 0.9% w/v NaCl solution (saline). All animals were killed 3 h after treatment and the brains were quickly removed, rinsed in cold saline, blotted, chilled and dissected on an ice-cooled plate. From each, the cerebellum was discarded, and the remaining brain tissue was dissected as described by Glowinski & Iversen (1966) into two portions referred to as the hindbrain (pons and medulla oblongata) and forebrain-midbrain (rest of brain). Labelled 5-HT and its major deaminated metabolite, 5-hydroxyindoleacetic acid (5-HIAA) were extracted essentially by the methods of Maickel, Cox, Saillant & Miller (1968) and Curzon & Green (1970), respectively. The radioactivity of sample portions (0.2 ml) was determined using 10 ml Aquasol scintillation fluid and counting in a liquid scintillation spectrometer. The counting efficiency for ^{14}C was 75%. The recovery of authentic 5-HT and 5-HIAA added to tissues prior to homogenization and carried through the procedure averaged 77.0 ± 1.7% (n = 6) and 80.0 ± 2.3% (n = 6), respectively. A slight overlap of 1.0% 5-HT in the 5-HIAA fraction and 1.3% 5-HIAA in the 5-HT fraction was observed. All data were corrected for appropriate recovery.

The method of Carlsson, Corrodi, Fuxe & Hökfelt (1969) was used to study the ability of benzoctamine and chlordiazepoxide to interfere with the uptake mechanism of the 5-HT neurones in the rat brain. Rats were injected i.p with two doses of α-ethyl-3-hydroxy-4-methylphenethyl-

amine (EMT), 25 mg/kg, 2 h apart. The animals were killed 2 h after the last dose of EMT. The drugs were injected i.p. 30 min prior to each dose of EMT, the second dose of the drug under test being half of the first. The whole brains were extracted for 5-HT (Maickel *et al.*, 1968) and the endogenous 5-HT determined fluorometrically (Bogdanski, Pletscher, Brodie & Udenfriend, 1956). Recovery of authentic 5-HT, added to brains prior to homogenization and carried through the method averaged 62.4 ± 2.5% (n = 12). All data were corrected for recovery. Percentage inhibition by drugs of uptake of 5-HT was calculated by the formula of Bruinvels (1971).

Chemicals and drugs employed in these studies were: [^{14}C]-5-HT (5-hydroxytryptamine bioxalate, specific activity 26.7 mCi/mM, New England Nuclear); α-ethyl-3-hydroxy-4-methylphenethylamine (EMT), (Aldrich Chemical Co., Inc.); chlordiazepoxide hydrochloride (Librium Hoffmann-LaRoche Ltd); benzoctamine hydrochloride (Tacitin, Ciba-Geigy Corp.). The latter two drugs were gifts from the respective companies.

Results

Both benzoctamine and chlordiazepoxide significantly elevated the concentrations of [^{14}C]-5-HT in the forebrain area, whereas only chlordiazepoxide produced this effect in the hindbrain (Table 1). The concentrations of [^{14}C]-5-HIAA, the major deaminated metabolite of 5-HT, were significantly increased in both areas of brain after each of the drugs.

EMT injected alone into rats caused a significant depletion of endogenous 5-HT (Table 2). Both benzoctamine at the higher dose (20 mg/kg followed by 10 mg/kg) and chlordiazepoxide at both doses examined, reduced the EMT-induced decrease of brain 5-HT. However, interpretation is difficult because the drug EMT combinations caused some hypothermia. Chlordiazepoxide alone at the higher dose caused a slight increase in the concentration of 5-HT.

Discussion

Intraventricular injections of labelled 5-HT have been used to study the effects of drugs on the central metabolism and transport of exogenous 5-HT (Palaic, Page & Khairallah, 1967; Schildkraut, Schanberg, Breese & Kopin, 1969). It appears that a portion of the intraventricularly-injected amine enters 5-HT nerve terminals (Fuxe, Hökfelt, Ritzen & Ungerstedt, 1968) where it mixes with the endogenous stores (Aghajanian & Bloom, 1967) and may serve as tracer for brain 5-HT.

The results demonstrate that benzoctamine and chlordiazepoxide cause significant alterations in 5-HT metabolism in the rat brain. In animals receiving the drugs, the concentrations of [^{14}C]-5-HT observed in the brain after intraventricularly-injected [^{14}C]-5-HT are elevated indicating decreased 5-HT turnover. These effects of benzoctamine and chlordiazepoxide on the fate of [^{14}C]-5-HT are consistent with those reported for diazepam (Chase *et al.*, 1970) and oxazepam (Wise *et al.*, 1972), minor tranquillizers related to chlordiazepoxide. The findings of a selective

Table 1 Effect of benzoctamine and chlordiazepoxide on [^{14}C]-total radioactivity (^{14}C-total), [^{14}C]-5-hydroxytryptamine (5-HT) and [^{14}C]-5-hydroxyindoleacetic acid (5-HIAA) concentrations in rat brain

	Treatment		
	0.9% w/v NaCl solution	*Benzoctamine (counts x 10^2/min/g)*	*Chlordiazepoxide*
(a) Forebrain-midbrain			
^{14}C-Total	107.4 ± 6.9	154.0 ± 10.0*	210.0 ± 31.8**
[^{14}C]-5-HT	44.3 ± 3.3	64.0 ± 5.1**	80.0 ± 10.0*
[^{14}C]-5-HIAA	51.3 ± 4.5	88.4 ± 8.8*	106.6 ± 20.0†
(b) Hindbrain			
^{14}C-Total	128.7 ± 5.3	156.7 ± 17.4	197.6 ± 17.5*
[^{14}C]-5-HT	84.3 ± 2.1	89.7 ± 11.2	118.8 ± 10.8**
[^{14}C]-5-HIAA	41.7 ± 1.1	62.7 ± 6.9**	72.0 ± 7.4*

Rats were injected intraventricularly with [^{14}C]-5-HT (0.35 μc) 10 min before the administration of drugs (20 mg/kg, i.p.) or 0.9% w/v NaCl solution. All animals were killed 3 h after treatment. Each group is mean with s.e. obtained from 5 rats.

Compared with control (0.9% w/v NaCl solution): * $P < 0.01$; ** $P < 0.02$; † $P < 0.05$.

Table 2 Effect of benzoctamine and chlordiazepoxide on the EMT-induced depletion of rat brain 5-hydroxytryptamine (5-HT).

Drug	First dose (mg/kg)	Brain 5-HT (μg/g) Drug alone	Drug +EMT	% Inhibition
Experiment No. 1				
0.9% w/v NaCl solution	–	0.46 ± 0.03	0.26 ± 0.02*	
Benzoctamine	20	0.49 ± 0.01	0.35 ± 0.01†	34
Chlordiazepoxide	20	0.54 ± 0.01**	0.39 ± 0.01†	36
Experiment No. 2				
0.9% w/v NaCl solution	–	0.52 ± 0.01	0.31 ± 0.01*	
Benzoctamine	10	0.53 ± 0.03	0.34 ± 0.1	11
Chlordiazepoxide	10	0.52 ± 0.01	0.36 ± 0.02††	24

Rats were injected with two doses of α-ethyl-3-hydroxy-4-methyl-phenylethylamine (EMT, 25 mg/kg, i.p.) 2 h apart. All animals were killed 2 h after the last dose of EMT. Drugs were injected, i.p., 30 min prior to EMT, the second dose being half the first. Each value is the mean with s.e. from 5-7 animals.
Compared with control 0.9% w/v NaCl solution: * $P < 0.001$; ** $P < 0.05$.
Compared with 0.9% w/v NaCl solution + EMT: † $P < 0.001$; †† $P < 0.05$.

decrease of cortical turnover after treatment with chlordiazepoxide (Lidbrink *et al.*, 1972) adds further support to an action of minor tranquillizers on 5-HT turnover.

Both drugs in the present study increased the levels of [^{14}C]-5-HIAA, the major deaminated metabolite of 5-HT. Similar effects on labelled 5-HIAA concentrations have been reported for diazepam (Chase *et al.*, 1970) and oxazepam (Wise *et al.*, 1972). However, if benzoctamine and chlordiazepoxide act only to decrease 5-HT turnover, the concentrations of 5-HIAA would be expected to decrease. As this was not observed, it may indicate that in addition to altering 5-HT turnover, both drugs interfere with the transport of 5-HIAA out of the brain. This suggestion has been considered for diazepam (Chase *et al.*, 1970).

A decrease in 5-HT turnover may be the result of one or more mechanisms such as a direct stimulation of 5-HT receptors, e.g. lysergic acid diethylamide (Andén, Corrodi, Fuxe & Hökfelt, 1968), inhibition of monoamine oxidase (Aghajanian, 1972), blockade of the 5-HT neuronal membrane uptake mechanism by such drugs as imipramine (Corrodi & Fuxe, 1969) or by a decrease in nerve impulse flow in 5-HT neurones (Andén, Fuxe & Hökfelt, 1966).

The first two mechanisms are unlikely to apply to benzoctamine and chlordiazepoxide since neither drug inhibits monoamine oxidase nor stimulates 5-HT receptors directly (Bein, 1970; Maître *et al.*, 1970; Randall, Heise, Schallek, Bagdon, Banziger, Boris, Moe & Abrams, 1961; Schallek, Schlosser & Randall, 1972). To determine whether these drugs affect the 5-HT neuronal membrane uptake mechanism, the method of Carlsson *et al.* (1969) was used. This utilizes EMT which has been shown to deplete central 5-HT stores by competing for the nueronal membrane process that recaptures nerve-released 5-HT. Drugs which block this 5-HT uptake mechanism also block the depleting action of EMT on brain 5-HT (Carlsson *et al.*, 1969). Both benzoctamine and chlordiazepoxide partially blocked the EMT-induced depletion of 5-HT. Thus, both drugs inhibit the 5-HT uptake mechanism and this could account, at least in part, for the decrease in 5-HT turnover observed, as postulated for certain tricyclic antidepressants (Corrodi & Fuxe, 1969). Blockade of the 5-HT uptake mechanism could lead to higher concentrations of 5-HT at the receptors, resulting in increased stimulation which, by a negative feedback mechanism, would lead to a decreased 5-HT release. However, diazepam, a congener of chlordiazepoxide does not block the uptake of intracisternally-injected labelled 5-HT in rat brain (Chase *et al.*, 1970), but decreases 5-HT turnover. Among the possibilities for this difference between the drugs is the observation that the combination of benzoctamine and/or chlordiazepoxide with EMT caused a lowering of body temperature. A similar observation for several major tranquillizing drugs combined with EMT has been reported (Carlsson *et al.*, 1969). Thus, the possibility that the blockade of the EMT-induced depletion of 5-HT may be a reflection of the hypothermia rather than a direct effect on the 5-HT re-uptake mechanism cannot be excluded.

The present findings demonstrate further the

uniqueness of the activity of benzoctamine. Benzoctamine, like the major tranquillizing drug chlorpromazine, accelerates the turnover of brain catecholamines (Maître *et al.*, 1970). In contrast, minor tranquillizers, like chlordiazepoxide, decrease catecholamine turnover (Taylor & Laverty, 1969; Corrodi *et al.*, 1971). Further, benzoctamine, unlike chlorpromazine, did not affect the neuronal uptake mechanism for noradrenaline (Maître *et al.*, 1970). In the present study, benzoctamine, like chlordiazepoxide, decreased 5-HT turnover. These results are thus different from those observed with chlorpromazine which does not affect the disappearance of intracisternally-injected labelled 5-HT (Schildkraut *et al.*, 1969); chlorpromazine slightly increased the concentrations of the labelled deaminated metabolites of [^{14}C]-5-HT, a result similar to that observed with benzoctamine and chlordiazepoxide in the present study.

Benzoctamine has been shown to exert anti-anxiety or anti-stress effects in man (Goldstein & Weiner, 1970). Recently, Wise *et al.* (1972) have postulated that 5-HT neurones may form part of a behavioural suppressant punishment system and that minor tranquillizers, which decrease the suppressant effects of punishment in animals, may do so by decreasing 5-HT turnover. It has been suggested that this effect might play a role in the anti-anxiety effects observed in man. The findings in the present study that benzoctamine, a new anti-anxiety drug, decreases the 5-HT turnover, as does the minor tranquillizer chlordiazepoxide, is consistent with this hypothesis.

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