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Dextromethorphan inhibits 5-hydroxytryptamine uptake by human blood platelets and decreases 5-hydroxyindoleacetic acid content in rat brain

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The effect of dextromethorphan on the uptake and metabolism of 5-hydroxytryptamine (5-HT) was studied in human blood platelets and in rat brain. In the concentration of 120 nM dextromethorphan inhibited the uptake of 5-HT (1 μM) into platelets by 50%. The corresponding concentrations of imipramine and methadone under similar conditions were 22 and 590 nM, respectively. Dextromethorphan (20 to 40 mg kg^{-1}) decreased the concentration of brain 5-hydroxyindoleacetic acid (5-HIAA) and the probenecid-induced accumulation of 5-HIAA time- and dose-dependently. However, dextromethorphan did not alter the pargyline-induced changes in brain 5-HT metabolism. Dextromethorphan-induced changes in brain 5-HT metabolism could arise from the inhibition of the reuptake of 5-HT into neurons.

Tricyclic antidepressants and pethidine in combination with monoamine oxidase (MAO) inhibitors can cause fatal toxic reactions such as excitation, hyperthermia and hypotension in man and in experimental animals e.g. in rabbits and in mice (Nymark & Møller-Nielsen, 1963; Loveless & Maxwell, 1965; Mustala & Jounela, 1966). These toxic reactions are at least partially mediated by increased activity of serotoninergic mechanisms in the brain (Rogers & Thornton, 1969; Jounela, 1970). Recently, Sinclair (1973) reported that dextromethorphan produces similar symptoms in rabbits pretreated with MAO inhibitors and fatalities have been reported following ingestion of dextromethorphan by patients treated with MAO inhibitors (Rivers & Horner, 1970). Moreover, Sinclair (1973) found that drugs that alter brain 5-hydroxytryptamine (5-HT) concentration altered the reactivity of rabbits towards the dextromethorphan-MAO inhibitor interaction.

Tricyclic antidepressants and pethidine block the reuptake mechanism for 5-HT in brain (Carlsson, Corrodi & others, 1969; Carlsson & Lindqvist, 1969) and in platelets (Todrick & Tait, 1969; Ahtee & Saarnivaara, 1973). Tricyclic antidepressants (Meek & Werdinius, 1970; Bruinvels, 1972) and pethidine (Ahtee & Saarnivaara, 1971, 1972) also decrease the concentration and/or the probenecid-induced accumulation of 5-hydroxyindoleacetic acid (5-HIAA) in the brain. One possible reason for the reduced 5-HIAA concentration and accumulation in brain produced by such 5-HT uptake blocking agents could be that these drugs prevent the access of 5-HT to mainly intraneuronal MAO. Therefore it was interesting to study if dextromethorphan, which interacts with MAO inhibitors in a manner similar to pethidine and tricyclic antidepressants, changes the 5-HT uptake and metabolism in the same way as do pethidine and the tricyclic antidepressants.

MATERIALS AND METHODS

Uptake of 5-HT by human blood platelets

Blood from healthy donors was mixed with one-ninth volume of 1·5% (w/v) solution of disodium edetate in 0·7% NaCl solution. The platelets were separated from plasma by centrifugation and resuspended in an equal volume of modified calcium-free Tyrode solution (Ahtee & Saarnivaara, 1973). Only polypropylene vessels and pipettes were used.

Uptake of 5-HT was studied by incubation of 1 ml duplicate samples of platelet suspension with or without [^{14}C]-5-HT of specific activity of 58 mCi mmol^{-1} (Radiochemical Centre, Amersham). Incubations were at 37°, in air with gentle shaking. The drugs (0·1 nM–0·1 mM) were added in a volume of 10 μl 10 min before 5-HT (1 μM) addition and the incubation was continued for 10 min. The samples were then immediately centrifuged and the supernatant decanted and traces of it remaining in the incubation tubes were removed with filter paper. The pellet was homogenized in distilled water by ultrasonification. The radioactivity was counted in a liquid scintillation counter Decem NTL 314.

Brain 5-HT and 5-HIAA concentrations

Sprague-Dawley female rats, 250–300 g, were used. The experiments were carried out at 20–22° between 9 a.m.– 1 p.m. The animals were decapitated and the brain rapidly removed and dissected. Whole brain except pineal and cerebellum was used for the spectrophotofluorimetric estimation of 5-HT and 5-HIAA (Ahtee, Sharman & Vogt, 1970). Recoveries of the standards through the method were for 5-HT 80% ± 2 (mean ± s.e.; 5 estimations) and for 5-HIAA 74% ± 2 (mean ± s.e.; 6 estimations). The data are not corrected for losses.

Drugs

The drugs used were gifts from following companies: dextromethorphan hydrochloride (F. Hoffman-La Roche CO.AG., Basle, Switzerland), imipramine hydrochloride (Geigy AG., Basle, Switzerland), probenecid (Merck Sharp & Dohme Ltd., Philadelphia, Pa), pargyline hydrochloride (Abbott Lab., North Chicago, Ill.), (±)-pethidine hydrochloride and (±)-methadone hydrochloride (Oy Star Ab, Tampere, Finland). Probenecid was dissolved in a small amount of 1N NaOH, diluted with 0·9% NaCl solution and neutralized with 0·1N HCl to pH 6·5–7·5. All the other drugs were dissolved in saline. The doses of drugs are expressed as base.

The statistical significance of differences between means was calculated by Student's *t*-test.

RESULTS

Effect of dextromethorphan on the uptake of 5-HT by human blood platelets

Dextromethorphan inhibited the uptake of 5-HT into human blood platelets *in vitro*. Concentrations causing 50% inhibition of 5-HT were determined by calculating regression lines for the per cent inhibition of 5-HT against drug concentration. Dextomethorphan (IC50(nM) = 120) under similar incubation conditions was about 5 times as potent as methadone (IC50 = 590) and about 80 times as potent as pethidine (IC50 = 9800) whereas imipramine (IC50 = 22) was about six times more active than dextromethorphan.

Effect of dextromethorphan on the 5-HT and 5-HIAA concentration of rat brain

Dextromethorphan (20 and 40 mg kg^{-1}, i.p. 2·5 h) did not alter the concentration of 5-HT in rat brain (5-HT $\mu g\ g^{-1}$: control 0·50 ± 0·03; 20 mg dose 0·46 ± 0·02, 40 mg dose 0·48 ± 0·03, means ± s.e. n = 3–5). However, at 20 mg kg^{-1} it decreased the concentration of 5-HIAA by 17% and at 40 mg kg^{-1} by 34% ($P < 0·01$) (5HIAA $\mu g\ g^{-1}$: control 0·29 ± 0·04; 20 mg dose, 0·24 ± 0·02; 40 mg dose 0·19 ± 0·01 means ± s.e. n = 3–5). It also retarded the probenecid-induced accumulation of 5-HIAA in rat brain (Table 1). This effect was time- and dose-dependent, so that the greatest inhibition occurred 1·5 h after 40 mg kg^{-1} of dextromethorphan when 5-HIAA accumulation was inhibited by 70%. Dextromethorphan (40 mg kg^{-1}, i.p. 1·75 h) did not alter the rate of the pargyline (75 mg kg^{-1}, i.p. 1·25 h) induced accumulation of 5-HT and reduction in 5-HIAA (5-HT $\mu g\ g^{-1}$: control 0·50 ± 0·03, pargyline alone 0·77 ± 0·01, pargyline + dextromethorphan 0·76 ± 0·04; 5-HIAA $\mu g\ g^{-1}$: control 0·29 ± 0·04, pargyline alone 0·18 ± 0·01, pargyline and dextromethorphan 0·16 ± 0·03, means ± s.e. n = 4–5).

Table 1. *Effect of dextromethorphan (i.p.: 0·5 h before probenecid) on the accumulation of 5-HIAA in the brain of rats treated with probenecid (200 mg kg^{-1}: i.p.; 1 or 2 h).* Means ± s.e. from 3 to 4 rats.

Dextromethorphan mg kg^{-1}	5-HIAA $\mu g\ g^{-1}$	
	1 h	2 h
0	0·46 ± 0·03	0·67 ± 0·05
20		0·51 ± 0·03*
40	0·34 ± 0·01**	0·46 ± 0·04**

* $P < 0·05$; ** $P < 0·01$.

DISCUSSION

These results show that dextromethorphan decreases the formation of 5-HIAA in rat brain. This effect could arise from the inhibition of the reuptake of 5-HT into neurons, whereby 5-HT would not reach the intraneuronal MAO. This possibility is supported by the finding that dextromethorphan prevented the uptake of 5-HT into human platelets. It is well documented that the 5-HT concentrating system of blood platelets is inhibited by drugs in the same way as that of neurons (Todrick & Tait, 1969; Paasonen, Ahtee & Solatunturi, 1971; Sneddon, 1973; Ahtee, Boullin & others, 1974). These experiments also suggest that dextromethorphan does not decrease the synthesis rate of 5-HT because it did not affect the pargyline-induced accumulation of 5-HT.

Thus these results suggest that dextromethorphan alters the brain 5-HT metabolism in a way similar to tricyclic antidepressants and pethidine (see introduction). The mechanism of action of imipramine and pethidine when producing toxic reactions in combination with MAO inhibitors is connected with 5-HT (Rogers & Thornton, 1969; Jounela, 1970). In addition, we recently found that, in contrast to pethidine, its analogues alphaprodine, anileridine or piminodine, which do not cause toxic reactions in combination with a MAO inhibitor (Jounela, Ahtee & Saarnivaara,

1971) do not alter the accumulation of 5-HIAA in brain (Ahtee & Saarnivaara, 1972). Therefore the present results support the suggestion of Sinclair (1973) that the toxic reactions of dextromethorphan when combined with MAO inhibitors are at least partially due to enhanced 5-HT responses.

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