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No correlation exists between antidepressant activity and the ability of 5-HT uptake inhibitors to interact with 5-HT receptors of the rat stomach fundus strip

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Several 5-HT uptake inhibitors, established and potential antidepressant drugs, were tested for their ability to counteract contractions of the rat isolated stomach fundus strip induced by 5-HT and $BaCl_2$. Of 12 inhibitors tested, only doxepine, amitriptyline, clomipramine, imipramine, Ro 11-2465 (cyan-imipramine), citalopram and fluvoxamine antagonized the contraction induced by 10^{-6} M 5-HT with IC50 values below 10^{-4} M. Amitriptyline, doxepine and cyproheptadine, at concentrations inhibiting the effect of 5-HT, did not antagonize the strip contractions induced by 3×10^{-3} M $BaCl_2$, while the remaining compounds that antagonized 5-HT-induced contractions, also antagonized—with at least a similar potency—the contractions induced by $BaCl_2$. From among antidepressant compounds investigated, only doxepine and amitriptyline may be regarded as antagonists of the 5-HT receptor in the rat stomach strip.

It has often been speculated that antidepressant drugs may exert their beneficial effect in the clinic by facilitation of the 5-hydroxytryptamin(5-HT)-ergic transmission in the brain, since many of them are potent inhibitors of the uptake of 5-HT (Coppen 1967; Carlsson et al 1969; Lapin & Oxenkrug 1969). However, such effective antidepressants as mianserin, trazodone, amitriptyline, doxepine and iprindol can block the central effects of 5-HT-mimetics (Maj et al 1977, 1978, 1979; Baran et al 1979; Ögren et al 1979; Pawłowski et al 1980; Nagayama et al 1981). In attempting to save the 'serotonergic' theory of depression, Aprison et al (1978) and Ögren et al (1979) developed a new hypothesis called the 'Hypersensitive serotonergic receptor theory of human depression' (Aprison et al 1978; Ögren et al 1979; Nagayama et al 1981). According to that, a hypersensitive 5-HT-ergic receptor is responsible for the state of clinical depression and antidepressant drugs exert their salutary effect via blockade of this receptor and via deceleration of the turnover of 5-HT, which is connected with 5-HT uptake inhibition.

We have previously found that most of the 5-HT uptake inhibitors attenuate (especially, at higher concentrations) the contractile responses to 5-HT of isolated rat stomach fundus strip (Pawłowski et al 1980, 1981a). Interestingly, among the drugs investigated, those that were never proved to be effective antidepressants, i.e. fluoxetine and Org 6582, did not affect the responses. On the other hand, amitriptyline and doxepine, generally recognized antidepressant drugs, exerted a potent anti-5-HT effect (though still weaker than cyproheptadine).

The present study, was a detailed investigation to see if peripheral anti-5-HT properties may be regarded as characteristic of antidepressant drugs. We therefore tested the established antidepressants amitriptyline, imipramine, clomipramine and doxepine, potential antidepressants such as citalopram (Gottlieb et al 1980; Lindegaard Pedersen et al 1982), fluvoxamine (Doogan 1980; Feldmann & Denber 1982), Ro 11-2465 (Gasic et al 1982) and zimelidine (Åberg & Holmberg 1979; Coppen et al 1979; Åberg 1981; Montgomery et al 1981), and fluoxetine (Wong et al 1975), Org 6582 (Sugrue et al 1976) and femoxetine (Buus Lassen et al 1975), compounds which are still suspected but proved rather ineffective as antidepressant drugs (Ghose et al 1977).

MATERIALS AND METHODS

Preparation of the rat isolated stomach fundus strips

Experiments were carried out on rat stomach strips from decapitated male Wistar rats 180–250 g. The stomach was dissected and strips were prepared as described by Vane (1957). The strips were set up in a 10 ml organ bath containing Tyrode solution at 37 °C aerated with carbogen (95% O_2 – 5% CO_2). The composition (g litre^{-1}) of the Tyrode solution was: NaCl, 8·0; KCl, 0·2; $CaCl_2$, 0·2; $MgCl_2$, 0·05; NaH_2PO_4, 0·04; $NaHCO_3$, 1·0 and glucose 1·5. The 5-HT- or $BaCl_2$-induced contractions were recorded

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isotonically using Linear Motion Transducer Model St-2 (Phipps & Bird, Inc.) and Line Recorder TZ 21S (Laboratorni Pristroje Praha). One end of a strip was attached to a lever of the transducer loaded with 1 g weight. Before testing, the strips were allowed to equilibrate for 60 min. During this time, as well as during the experiments, the overflow of the Tyrode solution bathing the strips was 1 ml min^{-1}. The drugs investigated were dissolved in the Tyrode solution bathing the strips. 5-HT and $BaCl_2$ were added directly into the bath in a volume of 0·1 ml.

Evaluation of antagonism

In the first series of experiments the antagonistic effect of drugs against contractions induced by 10^{-6} M 5-HT was investigated. This concentration of 5-HT produced submaximal (70%) contraction of the control strip. Before addition of 5-HT into the bath, the strips were incubated for 30 min with different concentrations of investigated drugs (see Pawłowski et al 1981a). The IC50 values (the concentration inhibiting by 50% the strip contraction induced by 5-HT) with corresponding confidence limits at $P = 0{\cdot}05$ for each compound were calculated by regression analysis. For a few selected drugs (doxepine, amitriptyline, imipramine and cyproheptadine) the cumulative dose-response curves were constructed as described by van Rossum (1963). The ED50 (EC50) of 5-HT was determined for each dose-response curve and the logarithm (ED50 in presence of the antagonist/ED50 in absence of the antagonist-1; dose ratio-1) was plotted against the logarithm of the concentration of the antagonist (Arunlakshana & Schild 1959) and—for each antagonist—the slope of the plot was calculated by regression analysis. pA_2 and pD_2' values were calculated according to the method of Ariëns & van Rossum (1957) and van Rossum (1963).

To estimate the specificity of the anti-5-HT action of investigated drugs, the antagonism of the drugs against stomach strip contractions induced by $BaCl_2$ (3×10^{-3} M) was assessed. As it was established in preliminary experiments, this concentration of $BaCl_2$ contracts the fundus strip to the same degree as it does 1×10^{-6} M 5-HT. The drugs were tested at concentrations equal their respective IC50 values against 5-HT. The results are expressed as a per cent of inhibition of the contraction induced by 3×10^{-3} M $BaCl_2$.

Drugs

The drugs used were: amitriptyline hydrochloride (Polfa), citalopram hydrobromide (Lundbeck), clomipramine hydrochloride (Anafranil, Ciba-Geigy), cyproheptadine hydrochloride (Merck, Sharp & Dohme), doxepine hydrochloride (Pfizer), femoxetine hydrochloride (FG 4963, Ferrosan), fluoxetine hydrochloride (Lilly 110140, Eli Lilly and Co.), fluvoxamine maleate (Philips-Duphar B.V.), imipramine hydrochloride (Polfa), norzimelidine hydrochloride (Astra), Org 6582 ((±)1-8-chloro-11-anti-amino-benzo-(b)-bicyclo-(3.3.1)-nona-3,6a-(10a)-diene hydrochloride, Organon), Ro 11-2465 (cyan-imipramine hydrochloride, Hoffmann-La Roche), 5-hydroxtryptamine creatinine sulphate (5-HT) (Sigma), zimelidine dihydrochloride (Astra).

RESULTS

Inhibition of submaximal response to 5-HT

The anti-5-HT effects of the investigated drugs are shown in Table 1. Zimelidine, norzimelidine, femoxetine and Org 6582 did not affect the contractile action of 5-HT (IC50 $> 1 \times 10^{-4}$ M); the remaining antidepressant drugs antagonized the effect of 5-HT but their potency was 40–2000 times weaker than that of cyproheptadine. Among those drugs the most potent were doxepine and amitriptyline (see Table 1).

Influence of doxepine, amitriptyline, imipramine and cyproheptadine on the 5-HT dose-response curve

As shown in Fig. 1, the 5-HT log dose-response curve was shifted to the right in the presence of doxepine, amitriptyline, imipramine or cyproheptadine. This shift was concentration-dependent for all these drugs. Doxepine, imipramine and cyproheptadine, but not amitriptyline, also decreased the maximal

Table 1. The effect of various 5-HT uptake inhibitors and cyproheptadine on the contractile action of 5-HT (10^{-6} M) in the preparation of the isolated rat stomach fundus strip.

Compound*	IC50 (M)	95% Confidence limits (M)
Doxepine	$2{\cdot}0 \times 10^{-6}$	$4{\cdot}0 \times 10^{-7}$–$7{\cdot}9 \times 10^{-6}$
Amitriptyline	$1{\cdot}0 \times 10^{-5}$	$6{\cdot}3 \times 10^{-6}$–$1{\cdot}6 \times 10^{-5}$
Clomipramine	$1{\cdot}5 \times 10^{-5}$	$4{\cdot}0 \times 10^{-6}$–$6{\cdot}0 \times 10^{-5}$
Imipramine	$2{\cdot}0 \times 10^{-5}$	$1{\cdot}6 \times 10^{-6}$–$2{\cdot}5 \times 10^{-4}$
Ro 11-2465	$4{\cdot}3 \times 10^{-5}$	$3{\cdot}5 \times 10^{-5}$–$5{\cdot}0 \times 10^{-5}$
Citalopram	$5{\cdot}0 \times 10^{-5}$	$2{\cdot}5 \times 10^{-5}$–$1{\cdot}0 \times 10^{-4}$
Fluvoxamine	$5{\cdot}2 \times 10^{-5}$	$2{\cdot}8 \times 10^{-5}$–$9{\cdot}5 \times 10^{-4}$
Fluoxetine	$1{\cdot}0 \times 10^{-4}$	$5{\cdot}0 \times 10^{-5}$–$2{\cdot}0 \times 10^{-4}$
Femoxetine	$>1{\cdot}0 \times 10^{-4}$	
Org 6582	$>1{\cdot}0 \times 10^{-4}$	
Zimelidine	$>1{\cdot}0 \times 10^{-4}$	
Norzimelidine	$>1{\cdot}0 \times 10^{-4}$	
Cyproheptadine	$5{\cdot}0 \times 10^{-8}$	$6{\cdot}3 \times 10^{-9}$–$1{\cdot}3 \times 10^{-8}$

* Pre-incubation time = 30 min.

response. The pA_2 and pD_2' values of doxepine, amitriptyline, imipramine and cyproheptadine are listed in Table 2.

Inhibition of the response to $BaCl_2$ (3×10^{-3} M)

As shown in Fig. 2, 3×10^{-3} M $BaCl_2$ produced a contraction of the strip of similar size as did 1×10^{-6} M 5-HT. Amitriptyline, doxepine and cyproheptadine used in concentrations equal to their IC50 values towards 10^{-6} M 5-HT did not affect the contractions induced by 3×10^{-3} M $BaCl_2$ (Fig. 2, Table 3). Other drugs did inhibit the effect of $BaCl_2$ by about 50% or more (Table 3).

DISCUSSION

The main finding of this study is that the ability to antagonize the peripheral 5-HT receptor present in the rat stomach fundus strip (RSFS-5-HT receptor)

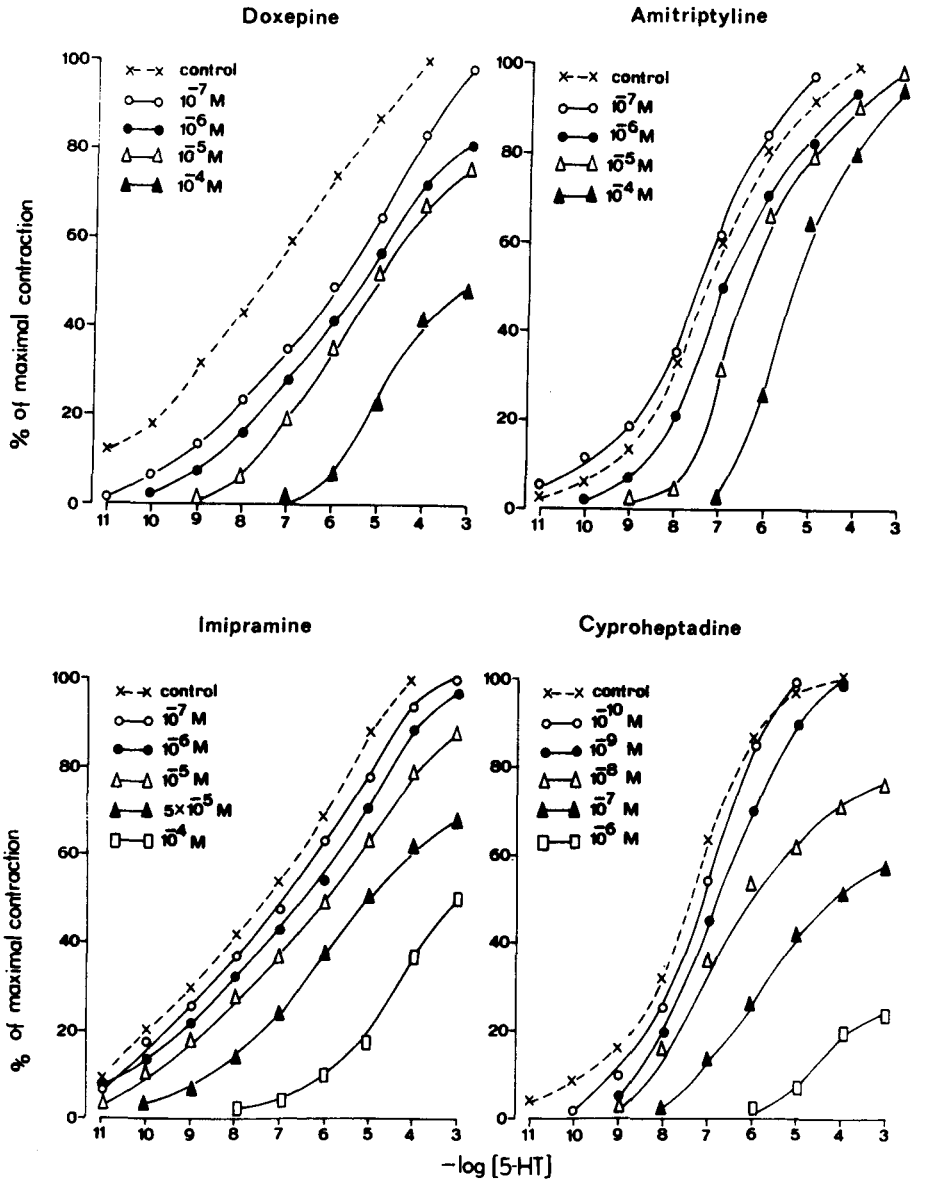

FIG. 1. Log dose-response curves of contractile responses of the rat stomach fundus strip to 5-HT in the absence (×---×) and presence (other symbols) of the increasing concentrations of doxepine, amitriptyline, imipramine or cyproheptadine. Each point is the mean value of at least 4 experiments.

is by no means a common characteristic of antidepressant drugs. From among a considerable array of established and potential antidepressant agents tested in this experiment, only amitriptyline was demonstrated to be a weak but competitive antagonist of this receptor, and possibly specific but non-competitive antagonism was found for doxepine. Both these compounds were, respectively, 200 and 40 times less potent in this respect than cyproheptadine, which also was found to be a non-competitive antagonist of RSFS-5-HT receptor.

Table 2. The inhibitory effect of doxepine, amitriptyline, imipramine or cyproheptadine (pre-incubation time = 30 min) on contractions of the rat stomach fundus strip caused by 5-HT.

			Plots of log (dose ratio-1) vs. log concentration of antagonist	
Compound	pA_2*	pD_2'*	Slope (b)	Correlation coefficient (r)
Doxepine	7·9 ± 0·30	5·3 ± 0·48	−0·48	−0·99
Amitriptyline	6·2 ± 0·26	3·6 ± 0·11	−1·11	−0·99
Imipramine	6·8 ± 0·31	4·4 ± 0·14	−0·46	−0·99
Cyproheptadine	9·2 ± 0·15	6·6 ± 0·01	−0·57	−0·98

* The values given in the Table represent the mean ± s.e.m. of at least 4 experiments.

Several other antidepressants inhibited the contractions of the preparation induced by 5-HT, as reported previously (Pawłowski et al 1980, 1981a), but as they were equally or more potent antagonists of the $BaCl_2$-induced contraction, they cannot be regarded as 5-HT antagonists (Clement 1981). Moreover, some potential antidepressants, including

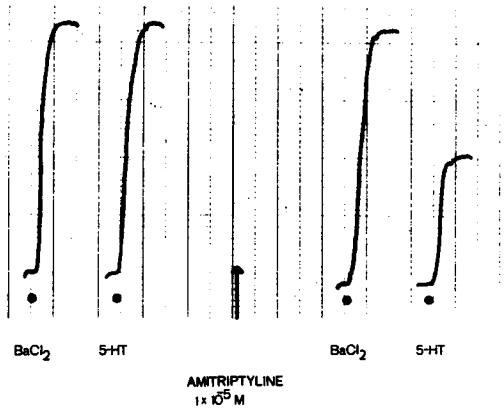

FIG. 2. The influence of amitriptyline (10^{-5} M, 30 min pre-incubation) on the $BaCl_2$ (3×10^{-3} M)- and 5-HT (10^{-6} M)-induced contraction of the rat stomach fundus strip. At the end of the first $BaCl_2$ or 5-HT trace (left) the preparation was washed out with normal Tyrode solution for 5 or 10 min, respectively. At the end of the second $BaCl_2$ trace the preparation was washed out (5 min) with Tyrode solution containing amitriptyline.

Table 3. The effect of various 5-HT uptake inhibitors and cyproheptadine on the contractile action of $BaCl_2$ (3×10^{-3} M) in the preparation of the isolated rat stomach fundus strip.

Compound[a]	Concentration used (M)	% of inhibition[b] (mean ± s.e.m.)
Doxepine	$5·0 \times 10^{-6}$	0
Amitriptyline	$1·0 \times 10^{-5}$	0
Clomipramine	$1·5 \times 10^{-5}$	53 ± 5·6
Imipramine	$2·0 \times 10^{-5}$	60 ± 4·6
Ro 11-2465	$4·3 \times 10^{-5}$	57 ± 10·6
Citalopram	$5·0 \times 10^{-5}$	41 ± 11·4
Fluvoxamine	$5·2 \times 10^{-5}$	58 ± 2·7
Fluoxetine	$1·0 \times 10^{-4}$	76 ± 12·8
Cyproheptadine	$1·0 \times 10^{-7}$	0

[a] Pre-incubation time = 30 min.
[b] At least 3 preparations for each drug were tested. '0' means: a change smaller than ±2·5%.

zimelidine (whose clinical antidepressant properties are fairly well documented: Åberg & Holmberg 1979; Coppen et al 1979; Åberg 1981; Montgomery et al 1981) did not affect the contractions of the preparation evoked by 5-HT.

In the view of the older and recent claims that anti-5-HT properties are relevant for antidepressant action (Aprison et al 1978; Ögren et al 1979; Nagayama et al 1981), the open question is if the model chosen was correct. Although the rat isolated stomach fundus strip is regarded as the best and most sensitive preparation to evaluate the anti-5-HT properties of drugs (Vane 1957; Offermeier & Ariëns 1966), the RSFS-5-HT receptor may be different from the central 5-HT receptors, on which antidepressant drugs were presumed to act.

Indeed, while the cerebral 5-HT receptors were characterized and divided into two subclasses differing between themselves in the respect of affinity to various ligands, and probably mediating different functions (Peroutka & Snyder 1979; Leysen et al 1981; Peroutka et al 1981), the RSFS-5-HT receptors could not be classified as either 5-HT_1 or 5-HT_2 receptors (Leysen et al 1981).

It should be added, however, that in another model for central anti-5-HT action, the inhibition of the stimulation of the hind limb flexor reflex in the spinal rat induced by 5-HT-mimetics (Maj et al 1976), the results obtained with several 5-HT uptake inhibitors, established or potential antidepressants, were similar to those reported here: amitriptyline and doxepine strongly inhibited the stimulation (Maj et al 1977, 1979; Pawłowski et al 1980), while other compounds were completely or almost completely inactive (Pawłowski et al 1980, 1981a; Maj et al

1982), and citalopram behaved even as an indirect weak agonist of 5-HT (Pawłowski et al 1981b).

Both the present and earlier (Pawłowski et al 1980, 1981a) results indicate in particular that the ability to inhibit the neuronal uptake of 5-HT, displayed by some antidepressant drugs, is unrelated to their ability to inhibit the 5-HT receptor, at least the RSFS-5-HT receptor and that involved in the flexor reflex. Moreover, the most effective antidepressant treatment, repeated electroconvulsive shock, results in an increase in the number of central 5-HT_2 receptors and facilitates the response to 5-HT-mimetics (Vetulani 1982).

It seems, therefore, that the recent claims that the antidepressant effect in the clinic is closely related to inhibition of 5-HT receptor (Nagayama et al 1981) is largely unsubstantiated; more detailed studies are required to find out if this can be true in the case of a specific subpopulation of central 5-HT receptors, different from the classical RSFS-5-HT receptor.

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