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Norzimelidine, a metabolite of a highly selective 5-hydroxytryptamine uptake inhibitor, can inhibit the uptake of noradrenaline in-vivo

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Zimelidine and norzimelidine were tested for their ability to counteract reserpine (2·5 mg kg^{-1})- or apomorphine (1–16 mg kg^{-1})-induced hypothermia and to potentiate TRH (40 mg kg^{-1})-induced hyperthermia in mice. Norzimelidine produced positive results in all three tests, behaving like a weak NA uptake inhibitor. Zimelidine was practically inactive. We conclude that the weak inhibitory effect of norzimelidine on the uptake of NA (in-vitro experiments) may be of importance for its pharmacological action and for the clinical action of zimelidine.

Zimelidine (H 102/09), a relatively new antidepressant drug of an already established clinical efficacy (Åberg & Holmberg 1979; Coppen et al 1979; Montgomery 1980; Åberg 1981; Montgomery et al 1981), is a fairly selective 5-hydroxytryptamine (5-HT) uptake inhibitor having practically no effect on the uptake of noradrenaline (NA) (for comparison with other drugs of this type see: Maître et al 1980; Hyttel 1982). Therefore its positive therapeutic effect (Åberg & Holmberg 1979; Coppen et al 1979: Montgomery 1980; Åberg 1981; Montgomery et al 1981) corroborates the 5-hydroxytryptaminergic theory of depression (Coppen 1967; Carlsson et al 1969; Lapin & Oxenkrug 1969). However, zimelidine, given chronically, produces effects similar to those observed after chronic treatment with highly selective NA uptake inhibitors and non-selective 5-HT uptake inhibitors that also inhibit the uptake of NA. Like desipramine, maprotiline, talsupram, amitriptyline and imipramine (Maj et al 1980, 1981; Pawłowski et al 1983), it potentiates clonidine-induced aggression in mice (Maj et al 1981) and, like desipramine, nortriptyline, amitriptyline, clomipramine and imipramine (Maggi et al 1980; Frazer & Lucki 1982), it decreases the number of β-adrenoceptors in the cerebral cortex of the rat (Ross et al 1981). Other highly selective 5-HT uptake inhibitors, such as citalopram, fluoxetine and fluvoxamine, are devoid of such effects (Maggi et al 1980; Maj et al 1981, 1982a; Hyttel et al 1983). In patients, chronic treatment with zimelidine decreases the concentration of the NA metabolite 4-hydroxy-3-methoxyphenyl glycol (HMPG) in the cerebrospinal fluid, the effect being the same as after chronic treatment with the NA uptake inhibitor – nortriptyline (Träskman-Bendz et al 1981). Actually, rat brain synaptosomes, incubated in plasma from patients treated for 2–4 weeks with zimelidine, take up much less [^{3}H]NA than control synaptosomes (Åberg-Wistedt et al 1981). These facts make the mechanism of the therapeutic action of zimelidine unclear and suggest that under in-vivo conditions zimelidine may be transformed into a metabolite (or metabolites) which is a stronger inhibitor of the uptake of NA than the parent drug.

The main metabolite of zimelidine (a bicyclic tertiary amine) is its desmethyl-derivative, norzimelidine (Ross & Renyi 1977; Bolvig Hansen et al 1980; Ross et al 1981) which, in contrast to the desmethyl-metabolites of tricyclic antidepressants (amitriptyline, imipramine, clomipramine), inhibits the uptake of 5-HT more potently than the parent compound (Ross & Renyi 1977; Åberg-Wistedt et al 1981; Hyttel 1982). After chronic treatment with zimelidine, norzimelidine accumulates in the body, reaching much higher concentrations in plasma than zimelidine (Åberg & Holmberg 1979; Coppen et al 1979; Bolvig Hansen et al 1980). Therefore norzimelidine (though never tested clinically) is thought to be an active metabolite of zimelidine and regarded as a selective 5-HT uptake inhibitor (Åberg & Holmberg 1979; Coppen et al 1979; Montgomery 1980). However, norzimelidine, besides having a more potent (4 times) action on the uptake of 5-HT, is 16 times more potent in inhibiting the uptake of NA (Åberg-Wistedt et al 1981; Hyttel 1982). Therefore the question arises whether norzimelidine may be regarded as a highly selective 5-HT uptake inhibitor, devoid of any practical effect on the uptake of NA.

The literature shows that there are no good direct tests for the evaluation of the effect of drugs on the uptake of NA in-vivo (Maître et al 1980). Therefore, to estimate the effect of zimelidine and norzimelidine on the uptake of NA in-vivo, we have used three pharmacological tests for NA uptake inhibitors which, in our opinion, are sensitive (and specific) enough to detect even negligible NA uptake-inhibiting properties of a compound. These tests were: antagonism to reserpine-induced hypothermia in mice (Slater et al 1979; Maj et al 1982b, 1983; Przegaliński et al 1983), antagonism to apomorphine (16 mg kg^{-1})-induced hypothermia in mice (Puech et al 1981; Pawłowski 1984) and potentiation of thyrotropin releasing hormone (TRH)-induced hyperthermia in mice (Desiles & Rips 1981; Pawłowski & Kwiatek 1983a, b).

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Materials and methods

The experiments were on male Albino-Swiss mice (25–35 g), that had free access to food and water until the beginning of the experiment. Before the experiment, the mice were adapted for 24 h to the conditions (temperature of 20 ± 1·5 °C for reserpine-induced hypothermia, or to a temperature of 21 ± 1 °C for apomorphine-induced hypothermia or TRH-induced hyperthermia). The rectal body temperature was measured with an Ellab T-3 thermometer at times specified in the text. The results are expressed as a change in the body temperature (Δt), in respect to the initial rectal temperature measured immediately before the injection of 5-HT uptake inhibitors. Inhibitors of the 5-HT uptake were administered intraperitoneally (i.p.) 20 h after subcutaneous (s.c.) injection of reserpine (2·5 mg kg^{-1}) or 30 min before s.c. injection of apomorphine (1 or 16 mg kg^{-1}) or i.p. injection of TRH (40 mg kg^{-1}). Each group consisted of 8–16 mice. The statistical evaluation was performed by Student's t-test.

The drugs given were: apomorphine hydrochloride (Sandoz), citalopram hydrobromide (H. Lundbeck & Co.), desmethyl-citalopram hydrochloride (H. Lundbeck & Co.), norzimelidine dihydrochloride (Astra), reserpine (Rausedyl; Gedeon Richter), TRH (thyrotropin releasing hormone, pyroglutamyl-histidyl-prolinamide; Scientific Research Division of the Institute of Chemistry of the University of Gdańsk), zimelidine dihydrochloride (Astra). Doses refer to the salts given. All drugs were dissolved in 0·9% NaCl (saline), except for reserpine which was taken from ampoules and dissolved in redistilled water, and then administered in a volume of 10 ml kg^{-1}.

Results and discussion

In the doses employed the 5-HT uptake inhibitors either did not affect body temperature in mice or decreased it (data not shown).

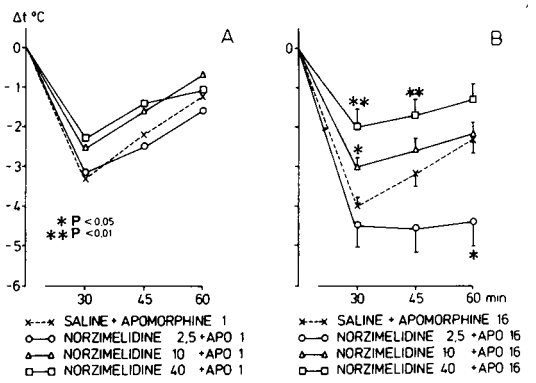

FIG. 1. The effect of norzimelidine (2·5–40 mg kg^{-1}) upon hypothermia induced by 1 (A) or 16 (B) mg kg^{-1} of apomorphine (APO) in mice kept at the ambient temperature of 21 ± 1 °C. Each symbol represents a mean change (Δt) in the rectal temperature (8 animals) ± s.e.m. * denotes a statistically significant result, $P < 0·05$, and **, 0·01, in comparison with the group receiving apomorphine alone (Student's *t*-test).

As shown in Table 1, norzimelidine (20–40 mg kg^{-1}) moderately antagonized the hypothermia induced by reserpine. In the same doses, used for comparison, the highly selective 5-HT uptake inhibitors zimelidine, citalopram and desmethyl-citalopram (Hyttel 1982) were inactive, or even demonstrated a tendency to decrease the body temperature already lowered by reserpine (Table 1). Norzimelidine (2·5–40 mg kg^{-1}) did not affect the hypothermia induced by a low dose (1 mg kg^{-1}) of apomorphine (Fig. 1A), easily antagonized by dopamine receptor antagonists (Puech et al 1981). However, norzimelidine (10–40 mg kg^{-1}) antagonized, in a dose-dependent manner, the hypothermia induced by 16 mg kg^{-1} of apomorphine (Fig. 1B), which is slightly counteracted by the dopamine receptor

Table 1. Effects of zimelidine, citalopram and their desmethyl-metabolites upon reserpine (2·5 mg kg^{-1})-induced hypothermia in mice kept at the ambient temperature of 20 ± 1·5 °C.

[a]Compound (mg kg^{-1})		[b]Initial temperature °C	Δt (°C ± s.e.m.) after 60 min	120 min	180 min	240 min
Saline		24·4 ± 0·96	0·1 ± 0·34	0·8 ± 0·44	1·4 ± 0·59	2·3 ± 0·70
Zimelidine	(20)	24·7 ± 0·88	−0·7 ± 0·31	−0·4 ± 0·30*	0·7 ± 0·46	2·0 ± 0·79
Zimelidine	(40)	25·3 ± 1·03	−0·3 ± 0·88	0·0 ± 0·98	0·7 ± 1·00	1·7 ± 1·10
Norzimelidine	(20)	24·9 ± 0·71	0·7 ± 0·41	1·7 ± 0·62	2·7 ± 0·79	4·4 ± 0·94
Norzimelidine	(40)	24·0 ± 0·69	0·7 ± 0·29	2·2 ± 0·45*	4·1 ± 0·67**	5·7 ± 0·83**
Saline		26·1 ± 0·86	1·4 ± 0·66	1·9 ± 0·81	2·5 ± 0·83	2·5 ± 0·73
Citalopram	(20)	23·5 ± 0·29	0·6 ± 0·54	0·6 ± 0·61	0·8 ± 0·53	1·1 ± 0·58
Citalopram	(40)	24·8 ± 0·57	0·9 ± 0·35	1·5 ± 0·55	2·0 ± 0·45	2·9 ± 0·61
Desmethyl-citalopram	(20)	26·5 ± 0·84	1·2 ± 0·40	2·0 ± 0·59	2·7 ± 0·69	3·3 ± 0·78
Desmethyl-citalopram	(40)	25·6 ± 0·60	1·9 ± 0·57	2·6 ± 0·75	3·4 ± 0·84	3·9 ± 0·97

[a] The drugs were administered 20 h after the reserpine injection.
[b] The rectal temperature of the mice before the reserpine injection was 37–38 °C. Each experimental group consisted of 10–12 mice.
*$P < 0·05$; **$P < 0·01$ (difference from the control (saline) group; Student's *t*-test).

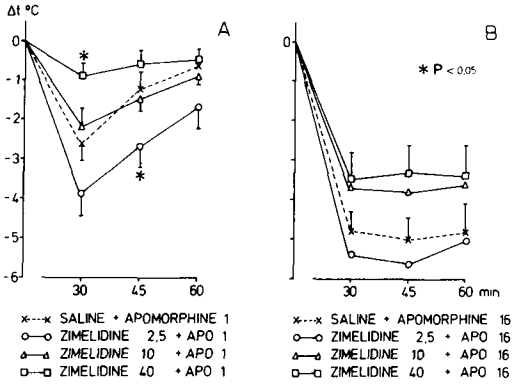

FIG. 2. The effect of zimelidine (2·5–40 mg kg^{-1}) upon hypothermia induced by 1 (A) or 16 (B) mg kg^{-1} of apomorphine (APO) in mice kept at the ambient temperature of 21 ± 1 °C. For other explanations see Fig. 1.

antagonists (Puech et al 1981) but effectively by NA uptake inhibitors (Puech et al 1981; Pawłowski 1984). The lowest dose of norzimelidine (2·5 mg kg^{-1}) tended to potentiate the apomorphine (16 mg kg^{-1})-induced hypothermia (Fig. 1B). In contrast to norzimelidine, zimelidine, when used in the highest dose (40 mg kg^{-1}), antagonized the hypothermia induced by 1 mg kg^{-1} of apomorphine (Fig. 2A), thereby exhibiting its weak anti-dopaminergic effect (Waldmeier 1982). Lower doses (2·5–10 mg kg^{-1}) of zimelidine either did not influence the apomorphine (1 mg kg^{-1})-induced hypothermia or even potentiated it (Fig. 2A). Zimelidine (2·5–40 mg kg^{-1}) did not antagonize the hypothermia induced by 16 mg kg^{-1} of apomorphine (Fig. 2B). Both zimelidine (5–20 mg kg^{-1}) and norzimelidine (2·5–10 mg kg^{-1}) potentiated TRH-induced hyperthermia (Fig. 3A, B). However, the effect of norzimelidine, already seen after a dose of 5 mg kg^{-1}, was at least 4 times stronger than that of zimelidine which itself

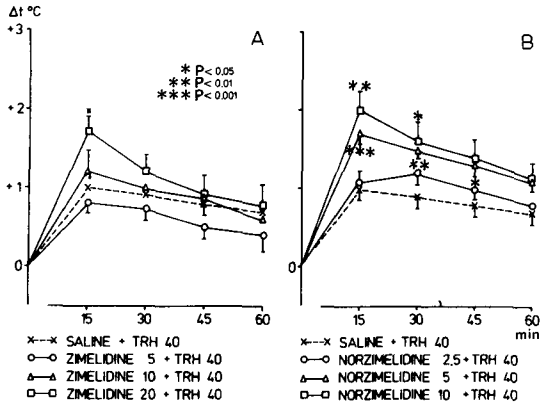

FIG. 3. The effect of zimelidine (5–20 mg kg^{-1}) (A) or norzimelidine (2·5–10 mg kg^{-1}) (B) upon hyperthermia induced by TRH (40 mg kg^{-1}) in mice kept at the ambient temperature of 21 ± 1 °C. Each experimental group consisted of 12–16 animals. For other explanations see Fig. 1. (***$P < 0.001$).

potentiated the TRH-induced hyperthermia only in doses of 20–40 mg kg^{-1} (Fig. 3A; Pawłowski & Mazela, unpublished).

The results indicate that norzimelidine, which is regarded as a clinically active metabolite of zimelidine (Åberg & Holmberg 1979; Coppen et al 1979; Montgomery 1980), differs pharmacologically from its parent compound. Norzimelidine antagonizes both reserpine- and apomorphine (16 mg kg^{-1})-induced hypothermia and potentiates TRH-induced hyperthermia (Table 1, Figs 1–3), whereas zimelidine is practically devoid of all such effects. Since norzimelidine inhibits the uptake of 5-HT in a more potent way than zimelidine (see introduction), it could be speculated that the difference in their pharmacological action reflects simply this fact. However, some 5-HT uptake inhibitors which are more potent than norzimelidine and highly specific such as citalopram, fluoxetine and fluvoxamine (Hyttel 1982; Hyttel unpublished results on fluvoxamine), neither antagonize the hypothermia induced by reserpine or apomorphine (1–16 mg kg^{-1}) nor potentiate TRH-induced hyperthermia (Claassen et al 1977; Slater et al 1979; Desiles & Rips 1981; Maj et al 1983; Pawłowski & Kwiatek 1983a, b; Pawlowski 1984; Table 1). Conversely, potent and highly selective NA uptake inhibitors, such as desipramine, maprotiline, oxaprotiline and talsupram (Hyttel 1982), are active in all the three tests in doses much lower than those used for norzimelidine in the present study (Slater et al 1979; Desiles & Rips 1981; Puech et al 1981; Maj et al 1983; Pawłowski & Kwiatek 1983a, b; Pawłowski et al 1983; Przegaliński et al 1983; Pawłowski 1984; Pawłowski unpublished). In addition, fluoxetine and citalopram do not potentiate, but rather inhibit, the antagonistic effects of desipramine or maprotiline on reserpine- or apomorphine-induced hypothermia (Maj et al 1983). These facts make inhibition of 5-HT uptake unlikely to be responsible for the positive effects of norzimelidine in our tests. The literature shows that positive results in the three tests we used are obtained only with those drugs that facilitate NA-ergic transmission, besides selective and non-selective NA uptake inhibitors (Slater et al 1979; Desiles et al 1980; Desiles & Rips 1981; Puech et al 1981; Maj et al 1982b, 1983; Pawłowski & Kwiatek 1983a, b; Pawłowski et al 1983; Przegaliński et al 1983; Pawłowski 1984), NA releasers, direct agonists of NA receptors and, perhaps, also inhibitors of monoamine oxidase (Frances et al 1979; Desiles et al 1980; Puech et al 1981; Przegaliński et al 1983; Pawłowski 1984). Drugs acting phramacologically through a different mechanism, e.g. dopamine receptor blockers or phosphodiesterase inhibitors, may, accidentally, produce positive results in one of the tests but not in all of them. For example, the dopamine receptor antagonists can attenuate apomorphine (1–16 mg kg^{-1})-induced hypothermia (Puech et al 1981) but they do not potentiate TRH-induced hyperthermia (Desiles et al 1980), while the phosphodiesterase inhibitors (rolipram, IBMX, Ro

20-1724) antagonize reserpine-induced hypothermia but not hypothermia induced by apomorphine (Przegaliński & Bigajska 1983).

The hypothetical effects of norzimelidine on monoamine oxidase, NA release and NA receptors have been previously excluded (Ross & Renyi 1977; Reveley et al 1979; Hall & Ögren 1981). Therefore the most likely explanation of the difference in pharmacological behaviour of norzimelidine from that of zimelidine observed by us, is the ability of norzimelidine to inhibit the uptake of NA in-vivo to a degree which may facilitate central NA-ergic transmission.

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