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COMMUNICATIONS

Ergometrine and 5-hydroxytryptamine binding sites in rat brain and myometrium

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Abstract—Functional studies suggest that ergometrine is a partial agonist involving 5-hydroxytryptamine (5-HT) receptors in rat uterus. Ergometrine displaced [^{3}H]5-HT from specific binding sites in rat brain, but did not displace [^{3}H]5-HT at functionally important concentrations in rat myometrium. These binding studies indicate that the agonist and antagonist actions of ergometrine in rat uterus arise from its initial interaction with binding sites other than those for 5-HT.

Ergometrine is widely used in obstetrics to contract the uterus post-partum to reduce haemorrhage. Recent functional studies suggest that ergometrine is a partial agonist involving 5-HT receptors in isolated rat uterus (Hollingsworth et al 1988). Ergometrine, as a spasmogen, was antagonised by methysergide and by ICI 169,369, a selective and competitive antagonist at 5-HT$_2$ receptors (Blackburn et al 1988; Hollingsworth et al 1988). Ergometrine was also a selective non-competitive antagonist of 5-HT in rat uterus. However, two observations suggest that these effects in rat uterus may not result from a simple interaction of ergometrine with the 5-HT$_2$ receptor. A latency of up to 1 min was observed between addition of ergometrine to the tissue bath and the spasmogenic response to ergometrine compared to a latency of less than 20 s with 5-HT. Also, methysergide produced greater antagonism of ergometrine than of 5-HT.

Recently Frenken & Kaumann (1987) have proposed that methysergide, a close structural analogue of ergometrine, interacts at an allosteric site of the 5-HT$_2$ receptor in vascular smooth muscle. The aim of this study was to determine if the actions of ergometrine in uterus involved a direct interaction with 5-HT receptors by assessment of the ability of ergometrine to displace [^{3}H]5-HT. Displacement of [^{3}H]5-HT from rat brain was used for comparison as there are many previous reports of specific [^{3}H]5-HT binding in rat brain (e.g. Bennett & Snyder 1976; Peroutka & Snyder 1979; Hoyer et al 1985).

Materials and methods

Binding assays. Binding studies were based on the method of Bennett & Snyder (1976). Whole brain (excluding cerebellum and pons-medulla) or endometrium-free uterus from non-pregnant female Sprague–Dawley rats (200–250 g) was homogenized in 0·05 M Tris-HCl and centrifuged at 35 000 RCF at 4°C for 20 min. The pellet was resuspended in 0·05 M Tris-HCl and incubated with [^{3}H]5-HT (3·4 nM; 537 GBq mmol^{-1}), in the presence of pargyline (1 μM), for 30 min at 37°C in the absence or presence of various concentrations of non-radioactive 5-HT or ergometrine. Bound and free [^{3}H]5-HT were separated by vacuum filtration using Whatman GF/C filters. Each assay was performed in triplicate. Non-specific binding was defined as the amount of [^{3}H]5-HT not displaced by 10 μM 5-HT.

Statistics. The data are expressed as means ± s.e.m. The differences between means were analysed by the Mann-Whitney U-test. A value of $2P < 0.05$ was considered significant.

Materials. 5-Hydroxtryptamine creatinine sulphate, ergometrine (ergonovine) maleate and pargyline were from Sigma Chemicals Co., Poole, Dorset, UK and [^{3}H]5-HT creatinine sulphate from Amersham International, Amersham, UK.

Results

Specific binding of [^{3}H]5-HT as a % of total binding was 56·4 ± 4·8% (n = 4) in brain and 64·3% ± 4·8% (n = 8) in myometrium. These values were not significantly different ($2P > 0.05$). 5-HT (3 nM to 10 μM) produced concentration-dependent displacement of [^{3}H]5-HT in brain with a pIC50 of 7·81 ± 0·26 (expressed as a $-\log_{10}$ M) (Fig. 1). The displacement curve was not of a simple sigmoidal shape but appeared to have an inflexion. The Hill coefficient was 0·60 ± 0·12 which was significantly less than 1·0 ($2P < 0.05$). 5-HT (30 nM to 10 μM) displaced [^{3}H]5-HT in myometrium with a pIC50 of 6·64 ± 0·11 and a Hill coefficient of 0·95 ± 0·12 which was not different from 1·0 ($2P > 0.05$). The pIC50 was significantly ($2P < 0.05$) lower than that in rat brain.

Ergometrine (10 nM to 10 μM) produced concentration-dependent displacement of [^{3}H]5-HT in rat brain with a pIC50 of 7·35 ± 0·29 (n = 5) (Fig. 1). The maximum displacement was 75·0 ± 7·0% of that produced by 5-HT. The Hill coefficient was

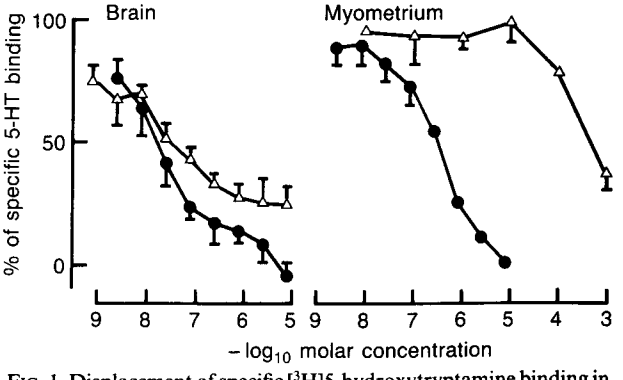

FIG. 1. Displacement of specific [^{3}H]5-hydroxytryptamine binding in brain and myometrium by 5-hydroxtryptamine (●——●) and ergometrine (△——△). Values are means ± s.e.m.

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$0{\cdot}35 \pm 0{\cdot}08$ which was less than 1·0 ($2P < 0{\cdot}01$). Ergometrine only produced significant displacement of [^{3}H]5-HT in myometrium at 1 mM (n = 5).

Discussion

The presence of specific [^{3}H]5-HT binding sites in rat brain confirms several previous reports (e.g. Bennett & Snyder 1976; Peroutka & Snyder 1979; Hoyer et al 1985). The pIC50 for 5-HT displacement of [^{3}H]5-HT in the current study (7·8) is similar to that reported previously (8, Bennett & Snyder 1976). This observation demonstrates the validity of the method used in the present study. Current ideas indicate the presence of multiple 5-HT binding sites in rat brain, designated $5HT_{1A}$, $5HT_{1B}$, $5HT_{1C}$ and $5HT_2$ (Hoyer et al 1985; Leff & Martin 1988) and may explain the Hill coefficient of less than 1 for 5-HT displacement of [^{3}H]5-HT.

This is the first report of a specific [^{3}H]5-HT binding site in rat myometrium. Although this is presumably the $5\text{-}HT_2$ receptor described in functional studies (Wigglesworth 1983; Bradley et al 1986; Hollingsworth et al 1988), further data are necessary for more conclusive support for this idea. A Hill coefficient not different from one suggests a single type of 5-HT binding site.

Ergometrine was able to displace [^{3}H]5-HT in rat brain and, therefore, we conclude that ergometrine can interact with at least one type of 5-HT binding site in this tissue. Previous studies have indicated that D-lysergic acid diethylamide and methysergide, both ergot alkaloids, can produce complete displacement of [^{3}H]5-HT in rat brain and interact with several 5-HT binding sites (Bennett & Snyder 1976; Peroutka & Snyder 1979; Peroutka 1986). By contrast, ergometrine only produced a maxium displacement of 75% of that produced by 5-HT which suggests that ergometrine interacts with fewer 5-HT sites than D-lysergic acid diethylamide or methysergide.

The most interesting observation was that ergometrine failed to displace [^{3}H]5-HT in myometrium except at the high concentration of 1 mM. This result is unlikely to be methodological as displacement could be detected in rat brain. It is necessary to relate this negative binding data to the previous positive functional data (Hollingsworth et al 1988). It is thus unlikely that the agonist and antagonist actions of ergometrine in rat uterus are due to a direct interaction with 5-HT receptors as spasmogen action was seen in the concentration range 30 nM to 1 μM and antagonist action from 0·1 μM, with 10 μM producing more than a 2000 fold antagonism of 5-HT.

It is possible that the agonist action of ergometrine is due to the compound releasing 5-HT (or other endogenous substances) from stores in rat uterus which is in turn an agonist(s) at the $5\text{-}HT_2$ receptor. However, this hypothesis would not explain the antagonist properties of ergometrine at the 5-HT receptor. Frenken & Kaumann (1987) have proposed that methysergide is an antagonist at the $5\text{-}HT_2$ receptor via an allosteric site. Leff & Martin (1988) have pointed out that methysergide is apparently a competitive antagonist at some $5\text{-}HT_2$ receptors and a non-surmountable antagonist at other $5\text{-}HT_2$ receptors. They have suggested that the variable nature of the antagonism can be explained by the antagonist slowly dissociating from receptors plus biochemical differences between tissues in the coupling between receptors and response. However, an interaction of ergometrine as a partial agonist at an allosteric site would explain both previous functional (Hollingsworth et al 1988) and present binding data. Clearly further functional and binding studies are necessary to help resolve which of the opposing hypotheses are more likely.

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The effect of repeated treatment with antidepressant drugs on the thyrotropin-releasing hormone (TRH)-induced hyperthermia in mice

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Abstract—The effect of acute (single dose) or repeated (twice daily, for 14 days) administration of 10 mg kg^{-1} p.o. of imipramine, amitriptyline, citalopram or mianserin has been examined on the hyperthermia induced by thyrotropin-releasing hormone (TRH) (40 mg kg^{-1} i.p., 2, or 2 and 72 h after single or last dose of antidepressants, respectively) in mice. Both imipramine and amitriptyline, given repeatedly, potentiated the TRH response, though the effect was observed 2 but not 72 h after the last dose of those drugs. Potentiation was also found after the single dose of imipramine or amitriptyline. On the other hand, citalopram and mianserin, administered either acutely or repeatedly, did not affect the TRH-induced hyperthermia.

Evidence indicates a link between antidepressant drugs and thyrotropin-releasing hormone (TRH) (Pecknold & Ban 1977; Nemeroff et al 1979). Others have recently reported that repeated antidepressant treatment reduces functional responses to the peptide. Sills & Jacobowitz (1987) found that long-term administration of desipramine or nialamide decreased the wet-dog shake response in rats induced by the TRH analogue MK-771(L-*N*-(2-oxopiperidin-6-ylcarbonyl)-L-histidyl-L-thiazolidine-4-carboxamide). Furthermore, Bennett et al (1986) showed that the hyperactivity, recovery from pentobarbitone-induced anaesthesia and reversal of both the pentobarbitone-induced hypothermia and decreased respiration—all evoked in rats by another TRH analogue, CG 3509(orotyl-histidyl-prolylamide) CG 3509—were significantly reduced following repeated treatment with amitriptyline.

Besides the above arousal effects, TRH causes hyperthermia in certain species, including mice (see Nemeroff et al 1979). Thus it was of interest to study the influence of repeated treatment with antidepressant drugs on this response to TRH. In the present paper we examined the effect of the two tricyclic antidepressants, imipramine and amitriptyline, the selective 5-hydroxytryptamine uptake inhibitor citalopram and the atypical antidepressant, mianserin.

Materials and methods

Male Albino Swiss mice (25–30 g), bought from licenced dealers, were kept at 21 ± 1°C, on a natural day-night cycle (spring), with free access to granulated rodent food (Bacutil) and tap water.

Imipramine hydrochloride (Polfa), amitriptyline hydrochloride (Polfa), citalopram hydrobromide (Lundbeck) or mianserin hydrochloride (Organon) were administered p.o. in a dose of 10 mg kg^{-1} acutely (single dose) or repeatedly (twice daily for 14 consecutive days). TRH (40 mg kg^{-1} i.p., synthesized in the Department of Chemistry, University of Gdańsk) was injected 2 h after the single dose, as well as 2 and 72 h after the last dose of the antidepressants. All the drugs were given as solution in 0·9% NaCl. Controls received the equivalent volume of saline.

The rectal body temperature was measured using an Ellab thermistor thermometer every 15 min for 1 h after TRH injection. The results are expressed as a change in the body temperature (Δt), with respect to the initial temperature measured immediately before the single or last dose of the antidepressant drugs.

Statistical significance of the results was assessed by Student's paired *t*-test.

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Results

TRH administered in doses of 10, 20, 40 and 80 mg kg^{-1} i.p. dose-dependently increased the rectal body temperature in mice with mean peak effects of 0·6, 0·8, 1·2 and 1·4°C, respectively, observed 15–30 min after its administration (results not shown). On the basis of these results the dose of 40 mg kg^{-1} of TRH was selected for the experiment with antidepressant drugs.

The hyperthermic response to TRH was potentiated in mice pretreated acutely or repeatedly with imipramine or amitriptyline, though in chronic experiment the potentiation was observed 2 but not 72 h after the last dose of antidepressants. Neither acute nor prolonged administration of citalopram or mianserin affected the TRH-induced hyperthermia (Fig. 1). None of the antidepressant drugs administered acutely, or repeatedly, affected the body temperature before TRH injection. The body temperature of mice, taken immediately before TRH injection, ranged from 36·5 to 37·1°C (results not shown).

Discussion

The present study has demonstrated that, of the four investigated antidepressant drugs, only imipramine and amitriptyline, given repeatedly, protentiated the TRH-induced hyperthermia in mice. However, this effect does not seem to result from their long-term administration, since it was observed only under drug treatment (i.e. 2 h after the last dose of the antidepressants), but not in the drug-free period (i.e. 72 h after their last administration), and since a similar potentiation—in accordance with other reports (Desiles & Rips 1980; Desiles et al 1980)—was also observed after a single dose of imipramine or amitriptyline. On the other hand, neither acute nor repeated treatment with the two other antidepressants, citalopram or mianserin, affected the response to TRH; the lack of effect of their single administration supports the results of other authors (Desiles et al 1980; Pawłowski & Nowak 1987). The potentiating effects of imipramine and amitriptyline, as well as the lack of effect of citalopram and mianserin, are in line with the results of other authors, indicating that among antidepressant drugs only noradrenaline uptake inhibitors, but not inhibitors of 5-hydroxytryptamine uptake or atypical antidepressants, potentiate the TRH-induced hyperthermia (Desiles et al 1980; Desiles & Rips 1981; Pawłowski & Kwiatek 1983).

Thus, our results show that the hyperthermic response to TRH in mice is not reduced by repeated treatment with the antidepressant drugs, though they were administered in doses which, according to Maj (1984), are sufficient to modify different behavioural responses mediated by α_1-adrenoceptors or dopamine receptors in the same species. This finding is in contrast to the literature data indicating that other functional responses to TRH (head twitch reaction, hyperactivity, arousal effects) are reduced following prolonged administration of antidepressants (Bennett et al 1986; Sills & Jacobowitz 1987). The reason for this