Metoclopramide antagonism of 5-hydroxytryptophan-induced 'Wet-Dog' shake behaviour in the rat

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Metoclopramide, a powerful antiemetic with central dopamine receptor blocking activity (Elliott, Jenner, Huizing, Marsden & Miller, 1977), has recently been shown to be a selective antagonist at receptors for 5-hydroxytryptamine (5-HT) on terminal cardiac sympathetic nerves (Fozard & Mobarok Ali, unpublished observations). Since methysergide is considered selective for smooth muscle 5-HT receptors (Douglas, 1975), it was of interest to compare the effects of these two peripheral antagonists on a model of central 5-HT activity, the 'Wet-Dog' shake behaviour (WDS) seen after 5-hydroxytryptophan (5-HTP) in the rat (Bedard & Pycock, 1977).

Male Sprague–Dawley rats (230–370 g) were injected (i.p.) with saline, with carbidopa (25 mg/kg) or with carbidopa plus methysergide or metoclopramide. Thirty min later (DL)-5-HTP (150 or 300 mg/kg) was administered i.p. The total number of Wet-Dog-like shakes occurring in the subsequent 140 min was recorded. In parallel experiments, whole brain 5-HT concentrations were estimated (Snyder, Axelrod & Zweig, 1965) 140 min after the time of injection of 5-HTP.

At 1 mg/kg, methysergide had no effect on WDS after carbidopa plus 5-HTP, but doses of 5, 10 and 100 mg/kg reduced the response in a dose-related manner. Metoclopramide (0.1–20 mg/kg) did not change WDS; after 40 and 100 mg/kg the response was essentially abolished. Methysergide (10 mg/kg) and metoclopramide (40 mg/kg) were chosen for further study.

Brain 5-HT concentrations were significantly increased by 5-HTP (150 mg/kg) and even further by pretreatment with carbidopa or by increasing the dose of 5-HTP to 300 mg/kg (Table 1). After methysergide (10 mg/kg) which blocked WDS, brain 5-HT concentrations were similar to those seen after carbidopa plus 5-HTP alone. After metoclopramide (40 mg/kg) on the other hand, inhibition of WDS was associated with blockade of the rise in brain 5-HT after carbidopa plus 5-HTP or 5-HTP alone (Table 1). Neither methysergide (10 mg/kg) nor metoclopramide (40 mg/kg) alone altered endogenous brain 5-HT concentrations.

The results point to a post-synaptic site of action for methysergide as a blocker of WDS. In contrast, inhibition of WDS by metoclopramide may result from prevention of the 5-HTP-induced rise in brain 5-HT concentrations.

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Table 1 Effects of methysergide and metoclopramide on 5-HTP-induced 'Wet-Dog' shake behaviour (WDS) and whole brain 5-HT concentrations in the rat

Treatment (mg/kg, i.p.)	*WDS (shakes in 140 min)*	*5-HT brain concentration (μg/g)*
Saline	1 ± 1 (3)	0.64 ± 0.03 (12)
5-HTP (150)	3 ± 1 (8)	1.08 ± 0.09 (7)
5-HTP (150) +carbidopa (25)	78 ± 9 (43)	1.76 ± 0.08 (8)
5-HTP (150) +carbidopa (25) +methysergide (10)	16 ± 4 (5)*	1.59 ± 0.18 (4)
5-HTP (150) +carbidopa (25) +metoclopramide (40)	6 ± 6 (6)*	0.98 ± 0.14 (8)*
5-HTP (300)	22 ± 6 (8)	1.66 ± 0.15 (6)
5-HTP (300) +metoclopramide (40)	0 (4)**	1.20 ± 0.11 (6)**

Values represent means ± s.e.mean; number of determinations in parentheses.
* $P < 0.05$ compared to 5-HTP (150 mg/kg) plus carbidopa (25 mg/kg).
** $P < 0.05$ compared to 5-HTP (300 mg/kg) alone.

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Baclofen enhances [^{3}H]-GABA release from rat globus pallidus *in vitro*

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The γ-aminobutyric acid (GABA) derivative β-parachlorophenyl-GABA (baclofen, Lioresal–CIBA–GEIGY) is believed to act as a GABAmimetic in the central nervous system (e.g. Andén & Wachtel, 1977) although this suggestion has been challenged (Davies & Watkins, 1974). We have explored the possibility that baclofen may enhance GABA-like transmission by a presynaptic action on GABA release. *In vitro* release of [^{3}H]-GABA was studied from rat globus pallidus, a region known to contain large numbers of GABA-like terminals. The methods used were essentially those of Srinivisan, Neal & Mitchell (1969). Pallidal cubes (0.2 mm) were prelabelled with transmitter (1 μCi/ml) and superfused at 1.5 ml/min with Krebs-bicarbonate buffer (37°C) containing aminooxyacetic acid (10^{-5} M). Results are expressed as fractional rate constants calculated from the total radioactivity recovered: significances were determined by Mann–Whitney 'U' test. The effect of depolarizing stimuli (50 mM K^+) and electrical stimulation (20 V, 1 ms, 50 Hz) or test drug (baclofen hydrochloride, DL-α,ε-diaminopimelic acid) on the efflux of radioactivity was studied.

Both electrical stimulation and K^+ (50 mM) enhanced the efflux of tritium. No change in the efflux of a [^{14}C]-sucrose space marker was seen during electrical stimulation. Electrically evoked increase of radioactivity was markedly reduced in the absence of calcium. Baclofen (400 μM) significantly increases efflux of radioactivity ($P < 0.02$), although baclofen (100 μM) alone produces only a small and non-significant enhancement. Aminocyclohexane carboxylic acid (20 μM), a selective competitive inhibitor of neuronal GABA transport (Bowery, Jones & Neal, 1976), significantly enhanced efflux of radioactivity, and addition of baclofen (400 μM) produced a further significant enhancement ($P < 0.05$). In the presence of β-alanine (20 μM), a competitive inhibitor of glial GABA transport (Iversen & Kelly, 1975), the spontaneous efflux was not significantly different from that produced by baclofen (400 μM) alone and subsequent addition of baclofen produced no further increase in efflux. Of the two stereoisomers only (+)-baclofen significantly ($P < 0.02$) enhanced radioactive efflux at 200 μM.

At baclofen (400 μM) also enhanced the efflux of [^{3}H]-β-alanine and [^{3}H]-diaminobutyric acid, presumed to be from glial and neuronal elements respectively (Iversen & Kelly, 1975).

DL-α,ε-Diaminopimelic acid, a neuronal depressant (Biscoe *et al.*, 1977) unrelated to GABA, had no effect on efflux of [^{3}H]-GABA at 1 mM.

These results suggest that baclofen may enhance GABA efflux in a specific manner unrelated to the direct neuronal depressant action of baclofen and may help to provide an explanation for the observed GABAmimetic actions of baclofen.

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