Effect of milacemide on extracellular and tissue concentrations of dopamine and 5-hydroxytryptamine in rat frontal cortex

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1 Milacemide is a glycine prodrug which is both an inhibitor and a substrate for monoamine oxidase-type B (MAO-B) and also an inhibitor of MAO-type A (MAO-A). Its effects on dopamine and 5-hydroxytryptamine (5-HT) metabolism in rat frontal cortex tissue and dialysate were evaluated.

2 Dialysate dopamine concentrations increased linearly and dose-dependently after milacemide administration (100, 200, 400 mg kg⁻¹, i.p.), peaking at 1 h. A concomitant dose-dependent decrease in dialysate 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations was observed but these changes were smaller (27% and 40% respectively) than the change in dopamine (125% after 400 mg kg⁻¹ milacemide).

3 Dialysate 5-HT was significantly increased only at 1.5 h after giving milacemide 400 mg kg⁻¹. Dialysate 5-hydroxyindoleacetic acid (5-HIAA) concentration was not affected.

4 Milacemide (400 mg kg⁻¹) at 1.5 h post-administration significantly increased frontal cortex tissue concentrations of dopamine and 5-HT; the percentage increase in dopamine being about four times that of 5-HT. Metabolite concentrations, including 5-HIAA, decreased. Changes in tissue and dialysate dopamine, DOPAC and HVA were approximately proportionate to each other.

5 The results are explicable in terms of an inhibition by milacemide of MAO-A.

Keywords: Dopamine; glycine prodrug; microdialysis; milacemide; monoamine oxidase; 5-hydroxytryptamine

Introduction

Milacemide (2-n-pentylaminoacetamide) is a drug with reported anticonvulsant (van Dorsser *et al.*, 1983; Houtkooper *et al.*, 1986; Roba *et al.*, 1986), antimyoclonic (Truong *et al.*, 1986), mood elevating and cognitive enhancing properties (Saletu *et al.*, 1986; Handelsmann *et al.*, 1989). It readily enters the brain, where it is metabolized by monoamine oxidase type B (MAO-B) to glycineamide (de Varebeke *et al.*, 1988; 1989; Semba *et al.*, 1991) and then to the inhibitory neurotransmitter glycine (Cavalier *et al.*, 1983; Christophe *et al.*, 1983). The drug also increases brain γ -aminobutyric acid (GABA; de Varebeke *et al.*, 1983) and blocks convulsions induced by bicuculline (van Dorsser *et al.*, 1983) which suggest a role for GABA in its pharmacological effects. However, according to a recent microionophoretic study, neither glycine nor GABA is involved in the depressant action of milacemide (Godfraind, 1990). Another possibility is that some of its actions may be mediated via dopamine or 5-hydroxytryptamine (5-HT) because it is an inhibitor as well as a substrate of MAO-B and also inhibits monoamine oxidase type A (MAO-A; de Varebeke *et al.*, 1989; Truong *et al.*, 1989b).

Acute treatment with milacemide is reported to increase tissue 5-HT in various rat brain regions including the cortex (Truong *et al.*, 1989a) and to have similar effects when given chronically to rhesus monkey (de Varebeke *et al.*, 1990). These findings are of clinical interest as milacemide opposes p,p'-DDT-induced myoclonus (Truong *et al.*, 1989a) which has been both proposed (Chung Hwang & Van Woert, 1978) and contested (Pratt *et al.*, 1985) as a rat model for human post-anoxic myoclonus. As intraventricular glycine alleviated the model (Truong *et al.*, 1986) and systemic treatment with the 5-HT precursor 5-hydroxytryptophan alleviates both the

model (Chang Hwang & Van Woert, 1978) and human post-anoxic myoclonus (Chadwick *et al.*, 1977) it was reasonable to suggest that milacemide might be of use in the human disorder (Truong *et al.*, 1986).

In the present study, the roles of 5-HT and dopamine in the neurochemical effects of milacemide and their possible implications for its behavioural and therapeutic properties were investigated by monitoring the effects of the drug on amine metabolism by means of a dialysis probe implanted in the frontal cortex of freely moving rats.

Methods

Male Sprague-Dawley rats (Charles River, Margate, Kent) weighing 250–300 g with free access to a normal laboratory diet (SDS R and M number 1 expanded, Scientific Dietary Services, Witham, Essex) and water were individually housed under a 12 h light–dark cycle (lights on 06 h 00 min).

Intracerebral microdialysis

Rats were anaesthetized with sodium pentobarbitone (60 mg kg⁻¹, i.p.) and dialysis probes implanted in the frontal cortex (3.2 mm anterior and 5.5 mm ventral to bregma, 2.5 mm lateral to the midline) according to Paxinos & Watson (1986). Concentric dialysis probes 4 mm long, 200 μ m diameter, were prepared as previously described (Hutson *et al.*, 1985) except that the internal glass capillary tubes were replaced by vitreous silica tubing (SGE, Milton Keynes) and Filtral 12 (Hospal, Rugby) was used for the dialysis membrane. The following day, artificial CSF (composition mM: NaCl 125, KCl 2.5, MgCl₂ 1.18 and CaCl₂ 1.26) was perfused through the probes at 2 μ l min⁻¹. Dialysate samples (60 μ l) were collected every 30 min for 3 h. Animals were then injected with milacemide (100, 200, or 400 mg kg⁻¹; Continental Pharma Inc., Belgium) or saline vehicle (0.9% NaCl) intraperitoneally and sampling continued for a further 3.5 h.

The first two dialysate samples were discarded and subsequent samples immediately analysed for dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA),

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5-HT and 5-hydroxyindoleacetic acid (5-HIAA). Fifty microlitres was directly injected and analysed by high performance liquid chromatography (h.p.l.c.) with electrochemical detection. An Altex Ultrasphere 3- μ m ODS column (4.6 mm $\times$ 7.5 cm; Beckman Ltd, U.K.) was used; mobile phase, 0.1 M phosphate buffer (pH 2.7) containing 18% methanol, 1 mM octanesulphonate and 0.1 mM EDTA. The flow rate was 1.3 ml min⁻¹. An ESA Coulochem electrochemical detector (model 5100A; Severn Analytical Ltd, U.K.) with a dual-electrode analytical cell (model 5100; Severn Analytical Ltd, U.K.) was used with electrode 1 set at 0.0 V, electrode 2 at 0.5 V and the guard cell (model 5020; Severn Analytical Ltd, U.K.) at +0.7 V. Limits of detection for dopamine, DOPAC, HVA, 5-HT and 5-HIAA were 0.30, 0.25, 1.00, 0.30 and 0.25 pmol ml⁻¹ respectively.

Frontal cortex monoamine and metabolite concentrations

In a separate experiment, rats were given milacemide (400 mg kg⁻¹, *n* = 8) or saline (*n* = 8) as before, decapitated 1.5 h later and the frontal cortex removed, weighed, and homogenized in 10 volumes of the mobile phase described above. After centrifugation at 16,000 *g* for 5 min, 5 μ l of the supernatant was analysed for dopamine, DOPAC, HVA, 5-HT and 5-HIAA by h.p.l.c. as before.

Statistics

Statistical analysis of the dialysis values was made by summary measures and analysed by one-way ANOVA followed by Dunnett's test. The Mann-Whitney U-test was used to compare tissue values in the different groups.

Results

Dialysates

Figure 1 shows dialysate dopamine, DOPAC and HVA in the frontal cortex after giving saline or milacemide (100, 200, 400 mg kg⁻¹). Results are expressed as percentage changes with respect to baseline. Dopamine values rose linearly and dose-dependently, peaking at 1 h after injection. Values for rats given milacemide (100 and 200 mg kg⁻¹) returned to baseline at 2 and 4 h after injection respectively but animals given 400 mg kg⁻¹ of the drug had values that were still significantly (*P* < 0.01) raised at 3.5 h after injection. DOPAC and HVA decreased dose-dependently but these changes were less striking (27% and 40% respectively after 400 mg kg⁻¹ milacemide) than the increases in dopamine (125%). Maximum changes of DOPAC were somewhat later (1.5–2.5 h) than those of dopamine (1 h); HVA changes were slightly more delayed (2–3 h).

A significant (*P* < 0.05) increase (25%) of 5-HT was observed only at 1.5 h after giving the highest dose of milacemide (Figure 2; Table 1). 5-HIAA values were unaffected.

Tissue

The effects of milacemide (400 mg kg⁻¹) on dopamine and 5-HT metabolism in frontal cortex tissue are shown in Table 1 together with values for dialysates from rats given the same dose in a separate experiment (see Figures 1 and 2). Tissue and dialysate was taken 1.5 h after drug administration. Milacemide significantly increased dopamine and 5-HT and significantly decreased their metabolites in the tissue, the percentage increase of dopamine being about 4 times that of 5-HT. Percentage changes of dopamine, DOPAC and HVA in dialysate were in general comparable but somewhat smaller than corresponding tissue changes. Ratios of concentrations in tissue to concentrations in dialysates were much larger for 5-HT than for dopamine and very much smaller for the metabolites than for the corresponding amines.

Figure 1 Dialysate dopamine (a), 3,4-dihydroxyphenylacetic acid (DOPAC; b) and homovanillic acid (HVA; c) before and after the administration of milacemide or 0.9% saline (vehicle). Values are mean of 6–7 rats and are expressed as a percentage of baseline; vertical bars show s.e.mean. Basal dialysate values for dopamine, DOPAC and HVA were 2.9 ± 0.2 , 170 ± 37 and 160 ± 27 pmol ml⁻¹ respectively (*n* = 26); saline (●); milacemide 100 mg kg⁻¹ (▼), 200 mg kg⁻¹ (■) and 400 mg kg⁻¹ (▲).

Discussion

The tissue/dialysate ratios for the amines and their metabolites (Table 1) are worth some comment. The much larger values for amines than for metabolites agree with previous data (Westerink *et al.*, 1987; Adell *et al.*, 1989) but the ratio for cortical dopamine is much lower than that for 5-HT and also much lower than that reported by Westerink *et al.* (1987) for striatal dopamine. This latter discrepancy could reflect both the higher firing rate of cortical than of striatal dopaminergic neurones (Bannon & Roth, 1983) and less efficient dopamine reuptake by the more diffuse cortical dopamine terminals. On the other hand, data on monkeys (Elchisak *et al.*, 1983) suggest that conjugation of cortical dialysate dopamine might influence the ratio in the opposite direction. The tissue/dialysate ratio for dopamine may also have been influenced by the perfusate Ca²⁺ concentration as we used 1.26 mM (which approximates the value in brain interstitial fluid; Hansen, 1985) but Westerink *et al.* (1987) used 3.4 mM which may have somewhat increased dopamine efflux (De Boer *et al.*, 1990). The lower cortical tissue/dialysate ratios for DOPAC and HVA than for 5-HIAA may however be explicable in terms of the removal of the dopamine metabolites from

Table 1 Effect of milacemide (400 mg kg⁻¹, i.p.) on dopamine, 5-hydroxytryptamine (5-HT) and their metabolites in frontal cortex tissue and dialysate

	Vehicle			Milacemide		
	Tissue	Dialysate	Tissue/dialysate	Tissue	Dialysate	Tissue/dialysate
Dopamine	512 ± 44	3.5 ± 0.9	146	1312 ± 355 ^a (+156%)	8.1 ± 1.8 ^b (+131%)	162
DOPAC	360 ± 36	120 ± 55	3.0	157 ± 23 ^a (-56%)	63 ± 24 ^b (-48%)	2.5
HVA	451 ± 17	123 ± 26	3.7	323 ± 26 ^a (-29%)	98 ± 18 ^c (-20%)	3.6
5-HT	2961 ± 171	1.6 ± 0.4	1851	4110 ± 278 ^a (+38%)	2.0 ± 0.3 ^c (+25%)	2055
5-HIAA	2208 ± 56	348 ± 74	6.3	1573 ± 76 ^a (-29%)	352 ± 74 (+1%)	4.5

DOPAC = 3,4-dihydroxyphenylacetic acid; HVA = homovanillic acid; 5-HIAA = 5-hydroxyindoleacetic acid. Values (pmol g⁻¹ or pmol ml⁻¹) are for rats 1.5 h after injection of saline vehicle (0.9% NaCl) or milacemide and are given as means ± s.e.mean, *n* = 6–8. Dialysis values are not corrected for recovery. Percentage differences from vehicle-treated animals are shown in parentheses. ^a *P* < 0.01 vs vehicle-treated group (Mann-Whitney U test); ^b *P* < 0.01, ^c *P* < 0.05 vs vehicle-treated group (one-way ANOVA followed by Dunnett's test).

cortical dialysates as conjugates (Elchisak *et al.*, 1977; Sarna *et al.*, 1984).

Although in the present study experiments were not undertaken to determine the possible contribution of diffusion of dopamine, 5-HT and metabolites into frontal cortex dialysate from other regions, the recent study of Schwartz *et al.* (1990) suggests that diffusion effects would be minimal. By use of two microdialysis probes, one placed in the medial hypothalamus and the other in the lateral hypothalamus, drug-induced increases in 5-HT at one site were not detected at the other site even though the probes were only 1 mm apart in the same side of the brain (Schwartz *et al.*, 1990).

Rats given milacemide (400 mg kg⁻¹) are reported to show increases of 5-HT in various brain regions including the frontal cortex and a relatively slight rise of striatal dopamine (Truong *et al.*, 1989a); rhesus monkeys given milacemide chronically had substantial increases of 5-HT in various parts of the cortex and of dopamine in the striatum (de Varebeke *et al.*, 1990). These changes were explained in terms of inhibition of MAO-B. In the present investigation, rats given milacemide (400 mg kg⁻¹) had moderate but significant increases and decreases of 5-HT and 5-HIAA respectively in cortical tissue and a small transient increase (significant only at 1.5 h post

administration) of 5-HT in cortical dialysates. Changes of dialysate 5-HT concentration are more indicative of changes at receptors than are brain tissue 5-HT changes. Thus, behavioural responses to the 5-HT releaser *p*-chloroamphetamine paralleled cortical dialysate but not tissue 5-HT (Hutson & Curzon, 1989).

The lack of an effect of milacemide (400 mg kg⁻¹) on dialysate 5-HT is consistent with the recent brief report (Brown *et al.*, 1991) that although patients with post-anoxic myoclonus respond therapeutically to the 5-HT precursor 5-hydroxytryptophan (Chadwick *et al.*, 1977) similar patients did not respond to milacemide (approx 12–34 mg kg⁻¹ day⁻¹). Nevertheless, as lower doses of milacemide than those used in the present study enhanced behavioural effects of 5-HT on rats (Truong *et al.*, 1989b) the drug may have greater effects on 5-HT concentrations at extra-cortical sites. Alternatively, the dopamine changes may conceivably have potentiated responses to 5-HT.

A previous dialysis study (Sleight *et al.*, 1988) has shown that extracellular 5-HT is substantially increased only if both MAO-A and MAO-B are inhibited. Thus, in the present study, the relatively large increases of both cortical tissue and dialysate dopamine in association with decreases of DOPAC and HVA and the absence of significant changes of 5-HT metabolism in rats given milacemide are explicable by inhibition of MAO-A. That dopamine is mainly deaminated by MAO-A is indicated by both brain tissue and dialysate studies (Green *et al.*, 1977; Fowler & Benedetti, 1983; Kato *et al.*, 1986; Waldmeier, 1987; Butcher *et al.*, 1990; Garrett & Soares de Silva, 1990). Figure 4 of Kato *et al.* (1986) is particularly relevant as interpolation reveals that when the specific MAO-A inhibitor clorgyline decreased rat striatal dialysate DOPAC by 44%, the decreases of HVA and 5-HIAA are 28% and 5% respectively; changes which are essentially identical with the effect of milacemide on dialysate amine metabolites in the present study (Table 1). Although de Varebeke *et al.* (1990) emphasize the role of MAO-B in the rise of dopamine in the caudate nucleus of rhesus monkeys given milacemide chronically and show that, under these conditions, MAO-B is inhibited more markedly than MAO-A, evidence is strong (see above) that rat brain DA is largely metabolized by MAO-A though MAO-B is probably involved when MAO-A is inhibited (Green *et al.*, 1977).

De Varebeke *et al.* (1990) propose milacemide as an adjuvant to L-DOPA in the treatment of Parkinson's Disease. This is worthy of further consideration since correction of the cortical dopamine deficiency which occurs in the disease (Scatton *et al.*, 1983) could oppose associated cognitive defects (Brozoski *et al.*, 1979; Simon *et al.*, 1980).

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Figure 2 Dialysate 5-hydroxytryptamine (5-HT; a) and 5-hydroxyindoleacetic acid (5-HIAA; b) in rat frontal cortex before and after the administration of milacemide or 0.9% saline (vehicle). Values are mean of 6–7 rats and are expressed as a percentage of baseline; vertical bars show s.e.mean. Basal dialysate values for 5-HT and 5-HIAA were 1.15 ± 0.12 and 233 ± 25 pmol ml⁻¹ respectively (*n* = 26): saline (●); milacemide 100 mg kg⁻¹ (▼), 200 mg kg⁻¹ (■) and 400 mg kg⁻¹ (▲).

References

- ADELL, A., SARNA, G.S., HUTSON, P.H. & CURZON, G. (1989). An *in vivo* dialysis and behavioural study of the release of 5-HT by *p*-chloroamphetamine in reserpine-treated rats. *Br. J. Pharmacol.*, **97**, 206–212.
- BANNON, M.J. & ROTH, R.H. (1983). Pharmacology of mesocortical dopamine neurons. *Pharmacol. Rev.*, **35**, 52–68.
- BROWN, P., THOMPSON, R.D., ROTHWELL, J.C., DAY, B.L. & MARSDEN, C.D. (1991). A therapeutic trial of milacemide in myoclonus and the stiff-person syndrome. *Mov. Disord.*, **6**, 73–75.
- BROZOSKI, T.J., BROWN, R.M., ROSVOLD, H.E. & GOLDMAN, P.S. (1979). Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science*, **205**, 929–932.
- BUTCHER, S.P., FAIRBROTHER, I.S., KELLY, J.S. & ARBUTHNOTT, G.W. (1990). Effects of selective monoamine oxidase inhibitors on the *in vivo* release and metabolism of dopamine in the rat striatum. *J. Neurochem.*, **55**, 981–988.
- CAVALIER, R., HERIN, M. & FRANZ, M. (1983). Metabolism of a new antiepileptic drug (milacemide) in the brain. *Arch. Int. Pharmacodyn. Ther.*, **270**, 169–170.
- CHADWICK, D., HALLETT, M., HARRIS, R., JENNER, P., REYNOLDS, E.H. & MARSDEN, C.D. (1977). Clinical biochemical and physiological features distinguishing myoclonus responsive to 5-hydroxytryptophan, tryptophan with a monoamine oxidase inhibitor and clonazepam. *Brain*, **100**, 455–487.
- CHRISTOPHE, J., KUTZNER, R., NGUYEN-BUI, W.D., DAMIEN, C., CHATELAIN, P. & GILLET, L. (1983). Conversion of orally administered 2-n-pentylaminoacetamide into glycineamide and glycine in the rat brain. *Life Sci.*, **33**, 533–541.
- CHUNG HWANG, E. & VAN WOERT, M.H. (1978). P,P'-DDT induced neurotoxic syndrome: experimental myoclonus. *Neurology*, **28**, 1020–1025.
- DE BOER, P., DAMSMA, G., FIBIGER, H.C., TIMMERMAN, W. DE VRIES, J.B. & WESTERINK, B.H.C. (1990). Dopaminergic-cholinergic interactions in the striatum. The critical significance of calcium concentrations in brain microdialysis. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **342**, 528–534.
- DE VAREBEKE, J.P., CAVALIER, R., DAVID-REMACLE, M. & YODIM, M.B.H. (1988). Formation of the neurotransmitter glycine from the anticonvulsant milacemide is mediated by brain monoamine oxidase B. *J. Neurochem.*, **50**, 1011–1016.
- DE VAREBEKE, J.P., NIEBES, P., PAUWELS, G., ROBA, J.C. & KORF, J. (1983). Effect of milacemide, a glycineamide derivative, on the rat brain γ -aminobutyric acid system. *Biochem. Pharmacol.*, **32**, 2751–2755.
- DE VAREBEKE, J.P., PAUWELS, G., BUYSE, C., DAVID-REMACLE, M., DE MEY, J., ROBA, J. & YODIM, M.B.H. (1989). The novel neuro-psychotropic agent milacemide is a specific enzyme-activated inhibitor of brain monoamine oxidase B. *J. Neurochem.*, **53**, 1109–1116.
- DE VAREBEKE, J.P., SCHALLAUER, E., RAUSCH, W.D., RIEDERER, P. & YODIM, M.B.H. (1990). Milacemide, the selective substrate and enzyme-activated specific inhibitor of monoamine oxidase B, increases dopamine but not serotonin in caudate nucleus of rhesus monkey. *Neurochem. Int.*, **17**, 325–330.
- ELCHISAK, M.A., COSGROVE, S.E., EBERT, M.H. & BURNS, R.S. (1983). Distribution of free and conjugated dopamine in monkey brain peripheral tissues and cerebrospinal fluid determined by high-performance liquid chromatography. *Brain Res.*, **279**, 171–176.
- ELCHISAK, M.A., MAAS, J.W. & ROTH, R.H. (1977). Dihydroxyphenylacetic acid conjugate: natural occurrence and demonstration of probenecid-induced accumulation in rat striatum, olfactory tubercle and frontal cortex. *Eur. J. Pharmacol.*, **41**, 369–378.
- FOWLER, C.J. & BENEDITTI, M.S. (1983). The metabolism of dopamine by both forms of monoamine oxidase in the rat brain and its inhibition by cimoxatone. *J. Neurochem.*, **40**, 1534–1541.
- GARRETT, M.C. & SOARES DA SILVA, P. (1990). Role of the type A and B monoamine oxidase in the formation of 3,4-dihydroxyphenylacetic acid (DOPAC) in tissues from the brain of rats. *Neuropharmacology*, **29**, 875–879.
- GODFRAIND, J.M. (1990). Microionophoretic study with milacemide, a glycine precursor, on mammalian central nervous system cells. *Br. J. Pharmacol.*, **100**, 119–125.
- GREEN, A.R., MITCHELL, B.D., TORDOFF, A.F. & YODIM, M.B.H. (1977). Evidence for dopamine deamination by both type A and type B monoamine oxidase in rat brain *in vivo* and for the degree of inhibition of enzyme necessary for increased functional activity of dopamine and 5-hydroxytryptamine. *Br. J. Pharmacol.*, **60**, 342–349.
- HANDELMANN, G.E., NEVINS, M.E., MUELLER, L.L., AANOUE, S.M. & CORDI, A.A. (1989). Milacemide, a glycine prodrug, enhances performance of learning tasks in normal amnesic rodents. *Pharmacol. Biochem. Behav.*, **34**, 823–828.
- HANSEN, A.J. (1985). Effect of anoxia on ion distribution in the brain. *Physiol. Rev.*, **65**, 227–244.
- HOUTKOOPER, M.A., VAN OORSCHOT, C.A.E.H., RENTMEESTER, T.W., HOPPENER, P.J.E.A. & ONKELINX, C. (1986). Double-blind study of milacemide in hospitalized therapy-resistant patients with epilepsy. *Epilepsia*, **27**, 255–262.
- HUTSON, P.H. & CURZON, G. (1989). Concurrent determination of effects of *p*-chloroamphetamine on central extracellular 5-hydroxytryptamine concentration and behaviour. *Br. J. Pharmacol.*, **96**, 801–806.
- HUTSON, P.H., SARNA, G.S., KANTAMANENI, B.D. & CURZON, G. (1985). Monitoring the effect of a tryptophan load on brain indole metabolism in freely moving rats by simultaneous cerebrospinal fluid monitoring and brain dialysis. *J. Neurochem.*, **44**, 1266–1274.
- KATO, T., DONG, B., ISHII, K. & KINEMUCHI, H. (1986). Brain dialysis: *In vivo* metabolism of dopamine and serotonin by monoamine oxidase A but not B in the striatum of unrestrained rats. *J. Neurochem.*, **46**, 1277–1282.
- PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Stereotaxic Coordinates* (second edition). San Diego: Academic Press.
- PRATT, J.A., ROTHWELL, J., JENNER, P. & MARSDEN, C.D. (1985). Myoclonus in the rat induced by P,P'-DDT and the role of altered monoamine function. *Neuropharmacology*, **24**, 361–373.
- ROBA, J., CAVALIER, R., CORDI, A., GORISSEN, H., HERIN, M., DE VAREBEKE, J.P., ONKELINX, C., REMACLE, M. & VAN DORSSER, W. (1986). Milacemide. In *New Anticonvulsant Drugs*. ed. Meldrum, B.S. & Porter, R.H. pp. 179–190. London: J. Libbey & Co.
- SALETU, B., GRUNBERGER, J. & LINZMAYER, L. (1986). Acute and subacute central nervous system effects of milacemide in elderly people. Double-blind placebo controlled quantitative electroencephalography and psychometric investigations. *Arch. Gerontol. Geriatr.*, **5**, 165–182.
- SARNA, G.S., HUTSON, P.H. & CURZON, G. (1984). Effect of methyl fluorodopa on dopamine metabolites: importance of conjugation and egress. *Eur. J. Pharmacol.*, **100**, 343–350.
- SCATTON, B., JAVOY-AGID, F., ROUGUIER, L., DUBOIS, B. & AGID, Y. (1983). Reduction of cortical dopamine, noradrenaline, serotonin and their metabolites in Parkinson's Disease. *Brain Res.*, **275**, 321–328.
- SCHWARTZ, D.H., HERNANDEZ, L. & HOEBEL, B.G. (1990). Serotonin release in lateral and media hypothalamus during feeding and its anticipation. *Brain Res. Bull.*, **25**, 797–802.
- SEMBA, J., RATNARAJ, N. & PATSALOS, P.N. (1991). Simple and rapid micro-analytical procedures for the estimation of milacemide and its metabolite glycineamide in rat plasma and cerebrospinal fluid by high performance liquid chromatography. *J. Chromatogr. Biomed. Applic.*, **565**, 357–362.
- SIMON, H., SCATTON, B. & LEMOAL, M. (1980). Dopaminergic A10 neurones are involved in cognitive function. *Nature*, **286**, 150–151.
- SLEIGHT, A.J., MARSDEN, C.A., MARTIN, K.F. & PALFREYMAN, M.G. (1988). Relationship between extracellular 5-hydroxytryptamine and behaviour following monoamine oxidase inhibition and L-tryptophan. *Br. J. Pharmacol.*, **93**, 303–310.
- TRUONG, D.D., GALLOWAY, M.P., PEZZOLI, G., JAMROZIK, Z. & FAHN, S. (1989a). Milacemide increases 5-hydroxytryptamine and dopamine levels in rat brain – possible mechanisms of milacemide antimyoclonic property in the p,p'-DDT-induced myoclonus. *Pharmacol. Biochem. Behav.*, **32**, 993–1001.
- TRUONG, D.D., DIAMOND, B., PEZZOLI, G., MENA, M.A. & FAHN, S. (1989b). Monoamine oxidase inhibitory properties of milacemide in rats. *Life Sci.*, **44**, 1059–1066.
- TRUONG, D.D., PRANZETELLI, M.R., JACKSON-LEWIS, V. & FAHN, S. (1986). Intracerebroventricular glycine blocks myoclonus induced by p,p'-DDT in the rat. *Ann. Neurol.*, **20**, 149.
- TRUONG, D.D., DE YEBENES, J.G., PEZZOLI, G., JACKSON-LEWIS, V. & FAHN, S. (1988). Glycine involvement in DDT-induced myoclonus. *Mov. Disord.*, **3**, 77–87.
- VAN DORSSER, W., BARRIS, D., CORDI, A. & ROBA, J. (1983). Anti-convulsant activity of milacemide. *Arch. Int. Pharmacodyn.*, **266**, 239–249.
- WALDMEIER, P.C. (1987). Amine oxidases and their endogenous substrates (with special references to monoamine oxidase and the brain). *J. Neurol. Trans. Suppl.*, **23**, 55–77.
- WESTERINK, B.H.C., DAMSMA, G., ROLLEMA, H., DE VRIES, J.B. & HORN, A.S. (1987). Scope and limitations of *in vivo* brain dialysis: a comparison of its application to various neurotransmitter systems. *Life Sci.*, **41**, 1763–1776.

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