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Comparative study of the effects of mianserin, a tetracyclic antidepressant, and of imipramine on uptake and release of neurotransmitters in synaptosomes

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The effects of mianserin, a tetracyclic antidepressant, on uptake and release of [^{3}H]noradrenaline (^{3}H-NA), [^{3}H]dopamine (^{3}H-DA), [^{3}H]-5-hydroxytryptamine(^{3}H-5-HT) and [^{3}H]γ-aminobutyric acid (^{3}H-GABA) in synaptosomes from different areas of the rat brain were investigated in a comparative study with the tricyclic antidepressant imipramine. Mianserin and imipramine were inhibitors of ^{3}H-NA uptake in the hypothalamus, but could not increase ^{3}H-NA release from noradrenergic nerve endings. This behaviour was similar to that of (+)-amphetamine. Both mianserin and imipramine had essentially no effect on ^{3}H-DA transport mechanisms in striatal synaptosomes. (+)-Amphetamine, in contrast, strongly affected both ^{3}H-DA uptake and release. Imipramine was stronger than mianserin in inhibiting ^{3}H-5-HT accumulation by striatal synaptosomes. In contrast, mianserin stimulated ^{3}H-5-HT release whereas imipramine was ineffective. Mianserin had virtually no inhibitory activity on ^{3}H-5-HT uptake by rat blood platelets. Imipramine was a modest inhibitor of ^{3}H-GABA accumulation by whole brain synaptosomes; mianserin had no effect. Both drugs did not alter ^{3}H-GABA release. These results indicate that mianserin interferes in a way different from that of tricyclic antidepressants with the neurotransmitter transport mechanisms at the presynaptic level.

Recently it has been reported by Goodlet & Sugrue (1974) that mianserin, a tetracyclic compound [1,2,3,4,10,14b-hexahydro-2-methyl-dibenzo[c,f]-pyrazino-[1,2-a]azepine monohydrochloride] with clinical efficacy as an antidepressant (Itil, Polvan & Hsu, 1972; Fell, Quantok & van der Burg, 1973), is essentially devoid of effect in conventional screening tests for antidepressant activity.

It is generally believed that antidepressant drugs affect the availability of neurotransmitters in the synapse by interfering with some of the mechanisms of neurotransmission, particularly those of release and reuptake of various neurotransmitter substances at the presynaptic level (Carlsson, Corrodi & others, 1969; Kannengiesser, Hunt & Raynaud, 1973; Bopp & Biel, 1974). Neurochemical studies centered on biogenic amine metabolism in the rat brain after drug treatment have indicated the existence of marked differences between the effects of mianserin and those of tricyclic antidepressants (Leonard, 1974).

A more direct approach to the study of events taking place at the presynaptic level may be given by *in vitro* experiments utilizing preparations of nerve endings isolated from different areas of the brain. We have compared mianserin and the tricyclic antidepressant imipramine for their ability to interfere with the uptake of various neurotransmitters by synaptosomes prepared from different areas of the rat brain. The effects of the two drugs on neurotransmitter release were also investigated utilizing a superfusion technique which prevents the reuptake of the substrates released from synaptosomes (Raiteri, Angelini & Levi, 1974a). The results indicate that mianserin has a neurochemical profile both quantitatively and qualitatively different from that of imipramine.

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METHODS

Preparation of synaptosomes

Male Wistar rats, 200–250 g, were decapitated, the brains rapidly removed and chilled in 0·32M sucrose and the striatum and hypothalamus were dissected from several brains, according to Glowinski & Iversen (1966), until about 400 to 600 mg of tissue was obtained from each area. Crude (P_2) or purified synaptosomes, prepared according to Gray & Whittaker (1962), were resuspended in 0·32M glucose to give a concentration of about 6–8 mg protein ml^{-1}.

The synaptosomal suspensions were diluted 1:10 with Krebs-Ringer medium (mM NaCl 128, $CaCl_2$ 2·7, $MgSO_4$ 1·2, KCl 5, Na_2HPO_4 5, tris-HCl buffer 10, at pH 7·3) and preincubated for 15 min at 37° in a rotary waterbath, in air.

To prevent the metabolism of the radioactive substrates, inhibitors of metabolism were added to the media used both in uptake and release experiments (12·5μM nialamide and 1mM ascorbic acid, in the experiments with biogenic amines; 10μM aminoxyacetic acid, in the experiments with GABA). Over 85% of the radioactivity was recovered in the form of the original compound, in the experimental conditions used.

Uptake experiments

After preincubation, a small volume (1/50 of the final volume) of a solution containing [7-^{3}H]-(−)-noradrenaline (8·1 Ci mmol^{-1}), [^{3}H]dopamine (2·3 Ci mmol^{-1}), [^{3}H]-5-hydroxytryptamine creatinine sulphate (10·7 Ci mmol^{-1}) or [2,3-^{3}H]-GABA (10 Ci mmol^{-1}) and varying concentrations of the test drugs was added to the incubation flasks. The final concentration of the various radioactive substrates is reported in the legends to the figures and to Table 1. After 10 min incubation, 0·4 ml aliquots of the synaptosomal suspension were collected and centrifuged as already described (Levi & Raiteri, 1973). The radioactivity of 3% perchloric acid extracts of the pellets was measured.

Release experiments

After preincubation, a small volume (1/100 of final volume) of a solution containing the desired concentration of the radioactive neurotransmitters (see legends to figures) was added to the incubation flasks, to prelabel the synaptosomes. After 10 min, 0·4 ml aliquots of the incubation mixture were transferred on 0·65μm Millipore filters, placed on the bottom of 8 parallel superfusion chambers

Table 1. *Inhibition of synaptosomal neurotransmitter uptake by mianserin and imipramine.* Control uptake values, expressed as pmol mg^{-1} protein per 10 min (Mean ± s.e.m. and number of experiments in parentheses) were: 40·0 ± 0·8 (n=9) for ^{3}H-NA in the hypothalamus; 189 ± 3·4 (n=10) for ^{3}H-DA in the striatum; 28·4 ± 0·7 (n=16) for ^{3}H-5-HT in the striatum and 39·0 ± 0·5 (n=8) for ^{3}H-GABA in the whole brain.

Substrate	Drug	Percent inhibition of uptake at drug concentrations (M): Drug concentration (M) 10^{-4}	$5\cdot10^{-5}$	10^{-5}	5.10^{-6}	10^{-6}	10^{-7}	10^{-8}	IC40*
^{3}H-NA	Mianserin			72·7		63·6	53·8	20·0	$5\cdot0 \times 10^{-8}$
(hypothal.)	Imipramine			76·5		71·1	63·7	47·2	$< 10^{-8}$
1×10^{-7}M	(+)-Amphetamine			81·3					
^{3}H-NA	Mianserin	70·1		35·2		23·1	12·0		$1\cdot5 \times 10^{-5}$
(whole brain)	Imipramine	78·4		42·1		23·9	10·2		$9\cdot0 \times 10^{-6}$
(2×10^{-7}M)									
^{3}H-DA	Mianserin	28·4	0	0					$> 10^{-4}$
(c. striatum)	Imipramine	46·4	4·5	0					$\sim 10^{-4}$
(1×10^{-7}M)	(+)-Amphetamine			82·4					
^{3}H-DA	Mianserin	51·1		12·5		7·5	0		$8\cdot0 \times 10^{-5}$
(whole brain)	Imipramine	72·2		24·2		13·2	6·7		$2\cdot5 \times 10^{-5}$
($3\cdot5 \times 10^{-7}$M)									
^{3}H-5-HT	Mianserin	79·2	69·4	45·9		7·5			$9\cdot0 \times 10^{-6}$
(c. striatum)	Imipramine			65·6	56·1	42·4	20·4		$8\cdot1 \times 10^{-7}$
(1×10^{-7}M)									
^{3}H-5-HT	Mianserin	75·1		31·6		14·0	5·7		$1\cdot3 \times 10^{-5}$
(whole brain)	Imipramine	86·2		70·1		50·8	21·7		$7\cdot0 \times 10^{-7}$
(4×10^{-7}M)									
^{3}H-5-HT	Mianserin			0		0			
(platelets)	Imipramine			85·0		43·6	22·1		$8\cdot0 \times 10^{-7}$
(1×10^{-7}M)	(+)-Amphetamine			21·5		3·0	0		$> 10^{-5}$
H^3-GABA	Mianserin	0		0		0	0		
(whole brain)	Imipramine	29·6		12·9		1·4	0		$> 10^{-4}$
(4×10^{-8}M)	Hydroxy-GABA	48·4		23·6		8·3	4·6		$6\cdot0 \times 10^{-5}$

* IC40 values were determined by incubating synaptosomal suspensions with a constant concentration of the radioactive substrate and varying concentrations of the drug, and are expressed as the molar concentration of drug giving 40% inhibition of uptake, as determined graphically from a logarithmic/probability plot.

thermostatically maintained at 37° (Raiteri & others, 1974a). The filters were washed with two 5 ml portions of medium at 37°, and superfused with glucose-containing oxygenated medium, at a rate of 0·5 ml min^{-1}. After 5–6 min, the superfusion medium was substituted with new medium containing the desired concentration of the various drugs. The superfusates were collected directly into liquid scintillation vials. The radioactivity remaining on the filters at the end of the superfusion period and that of the fractions collected was measured.

Experiments with blood platelets

Rat platelet-rich plasma was prepared by a method similar to that of Tuomisto (1974) for rabbit platelet-rich plasma. Rat blood was collected directly from the heart, under light ether anaesthesia, into a polypropylene cylinder containing 1/9 vol of 1·5% disodium edetate (EDTA) in 0·7% NaCl. The platelet-rich plasma was obtained by centrifugation for 20 min at about 150 *g*. 1 ml aliquots of the supernatant were preincubated at 37° for 10 min in a rotary waterbath. Then a small volume of Krebs-Ringer medium containing ^{3}H-5-HT (1×10^{-7}M final concentration) and varying concentrations of the test drugs was added and the incubation continued for 10 min. Aliquots (0·4 ml) of the incubation mixture were then filtered through 0·65 μm Millipore filters on a Multiple Membrane Filter (Yeda Research and Development Co., Rehovot, Israel) and washed within a few seconds under moderate vacuum with two 5 ml portions of substrate-free incubation medium at 37°. The radioactivity remaining on the filters was measured.

RESULTS

Uptake inhibition experiments

The results of the experiments on uptake inhibition are summarized in Table 1. Imipramine and mianserin (like (+)-amphetamine) were inhibitors of ^{3}H-NA uptake in synaptosomes from the hypothalamus. When tested with synaptosomes from whole brain, the two drugs showed a pattern qualitatively similar to that obtained with hypothalamic synaptosomes, although the inhibitory effect was lower with synaptosomes from whole brain than with hypothalamic nerve endings.

In the striatum, both imipramine and mianserin were poor inhibitors of ^{3}H-DA uptake. On the contrary, (+)-amphetamine strongly inhibited the accumulation of the radioactive amine. When tested with synaptosomes from whole brain, both imipramine and mianserin showed a moderate inhibitory activity, the former being stronger than the latter.

When the effects on ^{3}H-5-HT uptake in striatal synaptosomes were examined, imipramine was more effective than mianserin in inhibiting the uptake of the amine. Similar results were obtained when whole brain synaptosomes were used. (+)-Amphetamine was reported to be a relatively weak inhibitor of ^{3}H-5-HT uptake in whole brain synaptosomes (Wong, Horng & Fuller, 1973).

When rat blood platelets were used, no inhibition of ^{3}H-5-HT uptake could be detected with mianserin at a concentration of 2×10^{-5}M, whereas imipramine inhibited 40% of the amine uptake at a concentration of 8×10^{-7}M and showed maximal inhibition at 10^{-5}M.

Mianserin did not show any inhibition of ^{3}H-GABA uptake even at a concentration of 10^{-4}M, whereas imipramine significantly inhibited the accumulation of the radioactive amino acid at a concentration of 10^{-5}M.

Release experiments

The effects of mianserin, imipramine and *p*-tyramine on the release of ^{3}H-NA from hypothalamic synaptosomes are shown in Fig. 1. Both mianserin and imipramine were without effect on ^{3}H-NA release, when added to the superfusion fluid at a concentration of 10^{-5}M. *p*-Tyramine increased ^{3}H-NA release 39% during 6 min stimulation.

Fig. 2 shows that both mianserin and imipramine, at a concentration of 10^{-5}M, only slightly stimulated the release of ^{3}H-DA from striatal synaptosomes. Desipramine (not shown) was devoid of any stimulatory effect. In contrast, (+)-amphetamine caused a large increase in ^{3}H-DA release (102% during 6 min).

The effects of mianserin, imipramine and (+)-amphetamine on ^{3}H-5-HT release from striatal synaptosomes are shown in Fig. 3. Mianserin increased by 33% the release of the amine during 6 min of stimulation; imipramine was ineffective. No increase in the release of ^{3}H-5-HT was observed when superfusing with 10^{-5}M desipramine (not shown). (+)-Amphetamine was a strong stimulator of ^{3}H-5-HT release (100% increase over control during 6 min).

Fig. 4 shows that both mianserin and imipramine did not modify the spontaneous release of ^{3}H-GABA from whole brain synaptosomes. Unlabelled GABA (10^{-5}M) strongly enhanced (310%) ^{3}H-GABA release.

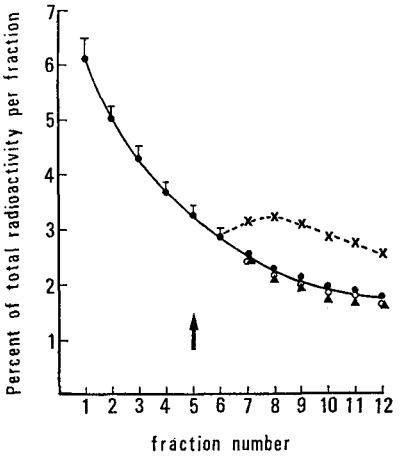

FIG. 1. Effect of mianserin ○—○, imipramine ▲—▲ and *p*-tyramine ×—× on ^{3}H-NA release from hypothalamic synaptosomes, control ●—●. Crude synaptosomal fractions (P_2 fractions) were washed once with 0·32M sucrose, resuspended in 0·32M glucose, diluted 1:10 in Krebs-Ringer medium and prelabelled with 10^{-7}M ^{3}H-NA. Then 0·4 ml aliquots of the suspension were placed on Millipore filters laying at the bottom of 8 parallel superfusion chambers and superfused at a rate of 0·5 ml min^{-1} with glucose-containing (10mM) oxygenated medium at 37°; fractions were collected every min. After 5 min, the superfusion medium was replaced either with identical medium (controls) or with medium containing the drug at the concentration of 10^{-5}M. The radioactivity in each fraction was measured and is expressed as percent of total radioactivity recovered (that is, total fractions plus filter). Each curve is the average of 4 experiments run in duplicate on 4 different days. The bars indicate the standard deviation. After the 5th fraction, standard deviations generally did not exceed ±0·1 and were not reported in this and in the following figures. Experiments (not presented) using purified hypothalamic synaptosomes gave identical results.

DISCUSSION

Mianserin and imipramine behaved similarly towards noradrenergic nerve terminals. They were inhibitors of ^{3}H-NA uptake in synaptosomes from the hypothalamus, an area which is particularly rich in noradrenergic terminals, but they were totally devoid of effect on ^{3}H-NA release. In this respect, the two drugs did not differ from (+)-amphetamine which also inhibited ^{3}H-NA uptake by hypothalamic nerve endings (Table 1), but was without effect on the release of the amine from superfused hypothalamic synaptosomes (Raiteri, Levi & Federico, 1974b; Raiteri, Bertollini & others, 1975b). The absence of any increase in ^{3}H-NA release, when synaptosomes were superfused with inhibitors of noradrenaline uptake, clearly indicates that reuptake of the spontaneously released ^{3}H-NA was completely prevented in the superfusion conditions used.

In the striatum, an area which is particularly rich in dopaminergic nerve endings, the uptake and release mechanisms for dopamine were virtually unaffected, both by mianserin and imipramine. These results and those obtained in the hypothalamus are in agreement with the results reported by Goodlet & Sugrue (1974) concerning the inhibition of (−)-metaraminol uptake in rabbit brain slices by desipramine and mianserin.

When synaptosomes from whole brain were utilized, both mianserin and imipramine inhibited ^{3}H-DA uptake more effectively than when striatal synaptosomes were used. This increased activity most likely represents inhibition of ^{3}H-DA uptake into noradrenergic nerve endings present in the synaptosomal preparation from whole brain. A similar reasoning applies to the results concerning ^{3}H-NA uptake inhibition in whole brain synaptosomes, where both mianserin and imipramine appeared to be less active than in the hypothalamus: in fact, a consistent portion of ^{3}H-NA is likely to be taken up by dopaminergic terminals (Snyder, Kuhar & others, 1970) in the synaptosomal preparation from whole brain and the 'dopamine pump' is almost insensitive to the drugs tested. The differences between the results obtained with synaptosomes from specialized brain areas and those obtained with whole brain synaptosomes indicate

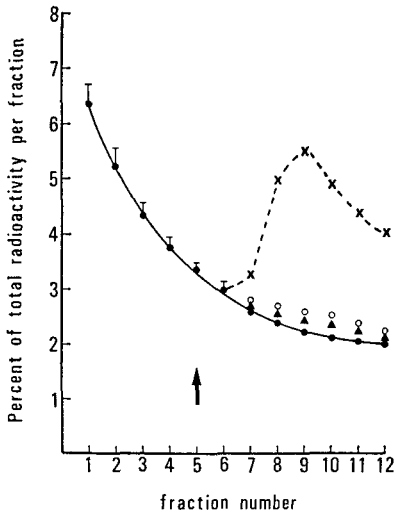

FIG. 2. Effect of mianserin ○—○, imipramine ▲—▲ and (+)-amphetamine ×—× on ^{3}H-DA release from striatal synaptosomes; control ●—●. For experimental details, see legend for Fig. 1. The concentration of ^{3}H-DA used for prelabelling the synaptosomes was 10^{-7}M. Each curve is the average of 4 experiments run in duplicate on 4 different days. Comparable results were obtained with purified striatal synaptosomes.

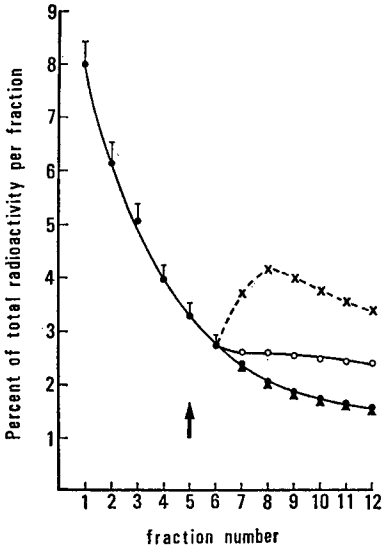

FIG. 3. Effect of mianserin ○—○, imipramine ▲—▲ and (+)-amphetamine ×—× on ^{3}H-5-HT release from striatal synaptosomes; control ●—●. For experimental details, see legend for Fig. 1. The concentration of ^{3}H-5-HT used for prelabelling the synaptosomes was 10^{-7}M. Each curve is the average of 4 experiments run in duplicate on 4 different days.

that the specificity of the synaptosomal labelling may be critical in uptake and release studies utilizing radioactive substrates.

The effects of mianserin on 5-HT transport were quantitatively and qualitatively different from those of imipramine. In uptake inhibition experiments imipramine was much stronger than mianserin, which was reported to be devoid of inhibitory activity on ^{14}C-5-HT uptake in slices from rat brain cortex (Leonard, 1974). On the other hand, mianserin was a stimulator of ^{3}H-5-HT release, whereas imipramine was without effect (Fig. 3). The selectivity of mianserin in stimulating 5-HT release deserves further investigation. That the effect observed with mianserin and (+)-amphetamine (Fig. 3) represents a 'true releasing effect' and not a mere inhibition of reuptake of the ^{3}H-5-HT spontaneously released is demonstrated by the fact that imipramine, which was the strongest inhibitor of 5-HT uptake among the three drugs tested, did not stimulate 5-HT release, whereas (+)-amphetamine, the weakest 5-HT uptake inhibitor, was the strongest 5-HT releaser.

The uptake of ^{3}H-GABA by whole brain synaptosomes was moderately inhibited by imipramine, in agreement with the literature (Iversen & Johnston, 1971). Mianserin was inactive at concentrations up to 10^{-4}M. The mechanism involved in the release of ^{3}H-GABA stimulated by unlabelled GABA (Fig. 4) is probably an homoexchange process (Levi & Raiteri, 1974; Raiteri, Federico & others, 1975a). An inhibition of the high affinity reuptake of ^{3}H-GABA spontaneously released by the unlabelled GABA added to the superfusion fluid should be excluded since, in the absence of sodium (a condition known to inhibit completely GABA high affinity uptake) (Bennett, Mulder & Snyder, 1974), ^{3}H-GABA release was unaffected (Raiteri & others, 1975a).

Blood platelets have been widely used as easily available models for nerve endings (Sneddon, 1973). The uptake of 5-HT in brain tissue and in blood platelets is inhibited by tricyclic antidepressants and since the concentration of drug giving 50% inhibition of 5-HT uptake in both tissues is identical, the molecular requirements of the 5-HT transport system in brain have been considered to be very similar or identical to those in blood platelets (Sneddon, 1973; Tuomisto, 1974). Our data seem to indicate that the uptake sites for 5-HT in rat striatal nerve endings are more accessible to mianserin than those in rat blood platelets. On the other hand, in the case of imipramine, our results are in agreement with the literature (Sneddon, 1973; Tuomisto, 1974).

In conclusion, the present study indicates that mianserin is likely to modulate in a way different

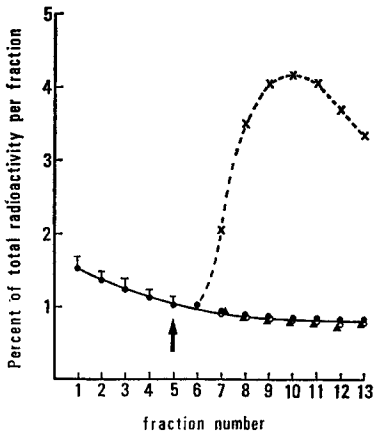

FIG. 4. Effect of mianserin ○—○, imipramine ▲—▲ and unlabelled GABA ×—× on ^{3}H-GABA release from the whole brain synaptosomes; control ●—●. Purified synaptosomes were prelabelled with ^{3}H-GABA at a concentration of 10^{-7}M, as described under Methods. For experimental details see legend for Fig. 1. Each curve is the average of 4 experiments run in duplicate on 4 different days.

from that of tricyclic antidepressants the mechanisms of release and reuptake of neurotransmitters at the presynaptic level.

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REFERENCES

BENNET, J. P., MULDER, A. H. & SNYDER, S. H. (1974). *Life Sci.*, **15**, 1045–1056.
BOPP, B. & BIEL, J. H. (1974). *Ibid.* **14**, 415–423.
CARLSSON, A., CORRODI, H., FUXE, K. & HOKFELT, T. (1969). *Eur. J. Pharmac.*, **5**, 367–373.
FELL, P. J., QUANTOK, D. C. & VAN DER BURG, W. J. (1973). *Eur. J. Clin. Pharmac.*, **5**, 166–173.
GLOWINSKI, J. & IVERSEN, L. L. (1966). *J. Neurochem.*, **13**, 655–664.
GOODLET, I. & SUGRUE, M. F. (1974). *Br. J. Pharmac.*, **52**, 430P.
GRAY, E. G. & WHITTAKER, V. P. (1962). *J. Anat.*, **96**, 79–87.
ITIL, T. M., POLVAN, N. & HSU, W. (1972). *Curr. Ther. Res.*, **14**, 395–413.
IVERSEN, L. L. & JOHNSTON, G. A. R. (1971). *J. Neurochem.*, **18**, 1939–1950.
KANNENGIESSER, M. H., HUNT, P. & RAYNAUD, J. P. (1973). *Biochem. Pharmac.*, **22**, 73–84.
LEONARD, B. E. (1974). *Psychopharmacologia* (Berl.), **36**, 221–236.
LEVI, G. & RAITERI, M. (1973). *Brain Res.*, **57**, 165–185.
LEVI, G. & RAITERI, M. (1974). *Nature Lond.*, **250**, 735–737.
RAITERI, M., ANGELINI, F. & LEVI, G. (1974a). *Eur. J. Pharmac.*, **25**, 411–414.
RAITERI, M., LEVI, G. & FEDERICO, R. (1974b). *Ibid.*, **28**, 237–240.
RAITERI, M., FEDERICO, R., COLETTI, A. & LEVI, G. (1975a). *J. Neurochem.*, **24**, 1243–1250.
RAITERI, M., BERTOLLINI, A., ANGELINI, F. & LEVI, G. (1975b). *Eur. J. Pharmac.*, **34**, 189–195.
SNEDDON, J. M. (1973). In: *Progress in Neurobiology*, p. 153. Editors: Kerkut, G. A. and Phillis, J. W. Oxford: Pergamon Press.
SNYDER, S. H., KUHAR, M., GREEN, A. I., COYLE, J. T. & SHASKAN, E. G. (1970). *Int. Rev. Neurobiol.*, **13**, 127–157.
TUOMISTO, J. (1974). *J. Pharm. Pharmac.*, **26**, 92–100.
WONG, D. T., HORNG, J. S. & FULLER, R. W. (1973). *Biochem. Pharmac.*, **22**, 311–322.