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Influence of acute and chronic chlorimipramine treatment on the 5-HT receptor-mediated modulation of acetylcholine release from the cerebral cortex of freely moving guinea-pigs

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1 Acetylcholine (ACh) release from the cerebral cortex of freely moving guinea-pigs, implanted with epidural cups, was studied.

2 A single dose of chlorimipramine (Cl-Imip, 10 mg kg^{-1}, s.c.), reduced the cortical ACh release both in normal and in chronically (10 mg kg^{-1} daily, s.c., for 14 days) Cl-Imip-treated guinea-pigs; the 5-HT$_3$ antagonist MDL 72222 (1 mg kg^{-1}, s.c.) antagonized this effect.

3 A single dose of Cl-Imip significantly reduced the effect of the 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-n-propylaminotetralin) (8-OH-DPAT, 0.1 mg kg^{-1}, s.c.), which nearly doubled the cortical ACh release in control animals. MDL 72222 restored to normal the response to 8-OH-DPAT reduced by the antidepressant.

4 A single dose of Cl-Imip did not change the inhibitory, MDL 72222-sensitive, effect induced by the 5-HT$_3$ agonist 2-methyl-5-hydroxytryptamine (2-methyl-5-HT, 500 μg, i.c.v.).

5 In chronically Cl-Imip-treated guinea-pigs, the facilitatory effect of 8-OH-DPAT was no longer present, while the inhibitory, MDL 72222-sensitive, effect of 2-methyl-5-HT was maintained.

6 These results indicate that the 5-HT$_{1A}$ receptor-mediated increase in ACh release is reduced by prolonged Cl-Imip treatment, while the 5-HT$_3$ receptor-mediated inhibition of ACh release is unaffected. The relevance of these findings to the antidepressant mechanism of Cl-Imip is discussed.

Keywords: Acetylcholine release; 5-HT receptors; antidepressants; chlorimipramine

Introduction

Antidepressant agents are known to affect the biochemistry and function of 5-hydroxytryptamine (5-HT) neurones in the brain (Heninger & Charney, 1987). The subdivision of 5-HT receptors into at least three broad classes, 5-HT$_1$, 5-HT$_2$ and 5-HT$_3$ (Bradley *et al.*, 1986), has focused interest on the role played by each subtype in the mechanism of action of antidepressant drugs. Because of the delayed onset of clinical efficacy with antidepressant drugs, most attention has been given to the adaptive changes in 5-hydroxytryptaminergic mechanisms following long-term antidepressant administration. Thus, behavioural (Goodwin *et al.*, 1985), electrophysiological (Blier *et al.*, 1988) and neuroendocrine (Wozniak *et al.*, 1989) paradigms, as well as changes in 5-HT receptor binding (Peroutka & Snyder, 1980), have been investigated.

5-HT modulation of acetylcholine (ACh) release from the cerebral cortex of freely moving guinea-pigs has been previously reported (Bianchi *et al.*, 1986). It has been shown that activation of 5-HT$_{1A}$ autoreceptors induces facilitation of ACh release (Siniscalchi *et al.*, 1990a; Bianchi *et al.*, 1990), while 5-HT$_3$ receptors inhibit ACh release (Bianchi *et al.*, 1990). Thus, the *in vivo* study of ACh release modulation provides a neurochemical correlate of 5-HT$_{1A}$ and 5-HT$_3$ receptor activation and may unmask possible changes in their functions, following antidepressant treatment. Part of the present findings have been the subject of a communication (Siniscalchi *et al.*, 1990b).

Methods

Guinea-pigs of either sex weighing 400–500 g, were treated with chlorimipramine (Cl-Imip), 10 mg kg^{-1} s.c. once a day, for 14 days. Another group of animals (controls) received only vehicle. On the 13th day, the animals were pretreated with midazolam, 7.5 mg kg^{-1} i.p. and anaesthetized with ketamine, 90 mg kg^{-1} i.p. A perspex cup (0.8 ml vol) was screwed into the right parietal bone and a stainless steel guide cannula for intracerebroventricular (i.c.v.) injection was also implanted (Beani *et al.*, 1978). Morphine, 2 mg kg^{-1} i.p., was administered on the day of the surgery. The experiments were carried out two days after surgery, i.e. 24 h after the last injection of Cl-Imip or vehicle. The release of ACh was measured by filling the epidural cups with 0.25 ml of Ringer solution containing physostigmine (0.3 mM), which was collected and renewed every 30 min. Generally, the experiments consisted of 5–6 collection periods so that any normal and drug-induced changes in ACh release could be reliably assessed.

The ACh present in the samples was bioassayed on tetrodotoxin-pretreated guinea-pig ileum (Beani *et al.*, 1978).

Drugs

The drugs used were: 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, RBI), 2-methyl-5-hydroxytryptamine (2-methyl-5-HT, Sandoz), 1αH,3α,5αH-tropan-3yl-3,5-dichlorobenzoate (MDL 72222) (Merrel Dow), midazolam (Roche), ketamine (Parke Davis), morphine HCl (Salars), chlorimipramine (Geigy) and tetrodotoxin (Sigma).

Statistical analysis

The statistical significance of the differences between the data before and after treatment, as well as between groups, was determined with ANOVA, followed by Student's *t* test for paired or non paired data.

Results

Acute administration of chlorimipramine

As shown in Table 1, Cl-Imip, 10 mg kg^{-1} s.c., acutely administered to freely moving guinea-pigs 30 min after a s.c. injection of saline, significantly reduced the cortical ACh release. Onset of the effect was slow, reaching its maximum 90 min

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Table 1 Effect of chlorimipramine (Cl-Imip, 10 mg kg^{-1} s.c.) on the release of acetylcholine (ACh) from the cerebral cortex of freely moving guinea-pigs pretreated (30 min before) with saline or MDL 72222 (1 mg kg^{-1} s.c.)

			ACh release				
					After Cl-Imip		
Pretreatment	No expts.	Before Cl-Imip (control)	30 min	60 min	90 min	120 min	150 min
Saline	5	147 ± 25	158 ± 34 (104)	122 ± 28* (81)	97 ± 17* (69)	102 ± 11* (76)	105 ± 11* (77)
MDL 72222	4	154 ± 23	155 ± 23 (102)	144 ± 24 (95)	144 ± 23 (95)	167 ± 26 (109)	—

* $P < 0.05$, significantly different from the controls, Student's t test for paired data.
Values are pmol cm^{-2} 30 min^{-1} ± s.e.mean. In parentheses the percentage of control values.

after administration, and lasted for over 2 h. Cl-Imip-induced inhibition was antagonized by the 5-HT$_3$ receptor antagonist, MDL 72222, 1 mg kg^{-1} s.c. (Table 1). MDL 72222 was devoid of any direct effect on ACh release i.e. 127 ± 11 pmol ACh cm^{-2} 30 min^{-1} before MDL 72222, and 127 ± 8, 144 ± 22, 120 ± 4 and 130 ± 12 pmol cm^{-2} 30 min^{-1} at 30, 60, 90 and 120 min respectively after MDL 72222, ($n = 5$).

Table 2 shows the facilitatory effect of the 5-HT$_{1A}$ agonist 8-OH-DPAT, 0.1 mg kg^{-1} s.c., on ACh release in control animals. Pretreatment with Cl-Imip, 10 mg kg^{-1} s.c. 30 min before 8-OH-DPAT, significantly reduced the facilitation induced by 8-OH-DPAT at 60 min (i.e. 90 min after Cl-Imip). MDL 72222 did not modify the ACh release increased by the 5-HT$_{1A}$ agonist (to 161% at 60 min, $n = 2$) and restored it to normal values in guinea-pigs acutely pretreated with Cl-Imip (Table 2).

In agreement with previous data (Bianchi *et al.*, 1990) 2-methyl-5-HT, 500 μg i.c.v., reduced cortical ACh release in control animals; this effect was antagonized by MDL 72222, 1 mg kg^{-1} s.c. (Figure 1a). Pretreatment of the guinea-pigs with Cl-Imip did not modify either the response to 2-methyl-5-HT, or the antagonism of this response by MDL 72222 (Table 3).

Chronic administration of chlorimipramine

As shown in Figure 2, in freely moving guinea-pigs, chronically (14 days) treated with Cl-Imip, 10 mg kg^{-1} daily s.c., 8-OH-DPAT, 0.1 mg kg^{-1} s.c., was no longer able to increase ACh release. However, 2-methyl-5-HT, 500 μg i.c.v., maintained its inhibitory, MDL 72222-sensitive effect (Figure 1b). In chronically treated animals, Cl-Imip, 10 mg kg^{-1} s.c., given 24 h after the last injection, still reduced ACh release: 138 ± 28 pmol ACh cm^{-2} 30 min^{-1} before Cl-Imip, reduced to 88%, 71%, 82% and 85% at 30, 60, 90 and 120 min respectively after Cl-Imip ($n = 5$). The latter values did not differ significantly from those for the saline-pretreated group (see Table 1).

Pretreatment with a single injection of Cl-Imip, 10 mg kg^{-1} s.c., did not modify the effects either of 8-OH-DPAT (Table 2), or of 2-methyl-5-HT (Table 3), challenged 24 h later.

Discussion

It is well known that Cl-Imip is a potent 5-HT uptake blocker, whereas its metabolite desmethylimipramine inhibits noradrenaline uptake (Rudorfer & Potter, 1989). The potencies and selectivities of antidepressant drugs as uptake inhibitors, on the other hand, do not appear sufficient alone to explain their clinical effect. In particular, blockade of amine uptake occurs rapidly (hours), while the clinical antidepressant effect has a long onset (weeks). Therefore, comparisons between the different effects produced by acute and chronic treatments may be useful in understanding the mechanisms involved in their antidepressant action.

ACh release from the cerebral cortex of freely moving guinea-pigs was reduced by acute administration of Cl-Imip. This effect was most likely due to the activation of 5-HT$_3$ receptors, since it was antagonized by MDL 72222. Cl-Imip may directly activate 5-HT$_3$ receptors, or, more likely, its action may be caused by increased levels of synaptic 5-HT: the delay in the onset of this Cl-Imip effect is compatible with the time required to attain uptake blockade (Rudorfer & Potter, 1989) and an increase of synaptic levels of 5-HT. Indeed, an inhibitory effect of 5-HT on ACh release, via 5-HT$_3$ receptors, has been demonstrated both *in vivo* (Bianchi *et al.*, 1990), and *in vitro* (Barnes *et al.*, 1989). When Cl-Imip was acutely administered before the 5-HT$_{1A}$ agonist 8-OH-DPAT, the facilitatory effect of the latter drug was significantly reduced.

Table 2 Effect of 8-hydroxy-2-(di-n-propylaminotetralin) (8-OH-DPAT, 0.1 mg kg^{-1} s.c.) on the release of acetylcholine (ACh) from the cerebral cortex of freely moving guinea-pigs pretreated (30 min before) with saline, chlorimipramine (Cl-Imip, 10 mg kg^{-1} s.c.) or Cl-Imip plus MDL 72222 (1 mg kg^{-1} s.c.)

			ACh release			
				After 8-OH-DPAT		
Pretreatment	No expts.	Before 8-OH-DPAT (control)	30 min	60 min	90 min	120 min
Saline[a]	8	125 ± 11	176 ± 16*** (141)	217 ± 22*** (178)	166 ± 22 (134)	116 ± 10 (94)
Cl-Imip[a]	6	106 ± 8	152 ± 15* (143)	142 ± 12° (135)	143 ± 12 (136)	135 ± 17 (129)
Cl-Imip[b]	3	132 ± 27	213 ± 12* (156)	199 ± 14* (164)	164 ± 23 (149)	159 ± 30 (135)
Cl-Imip[a] + MDL 72222	4	128 ± 6	170 ± 8* (134)	218 ± 17* (168)	158 ± 20 (122)	138 ± 22 (116)

Values are pmol ACh cm^{-2} 30 min^{-1} ± s.e.mean; in parentheses the percentage of control values. *$P < 0.05$; ***$P < 0.001$ significantly different from the corresponding controls, Student's t test for paired data. °$P < 0.05$ significantly different from saline and Cl-Imip + MDL 72222 groups, Student's t test for non paired data.
[a] 30 min before 8-OH-DPAT, [b] 24 h before 8-OH-DPAT.

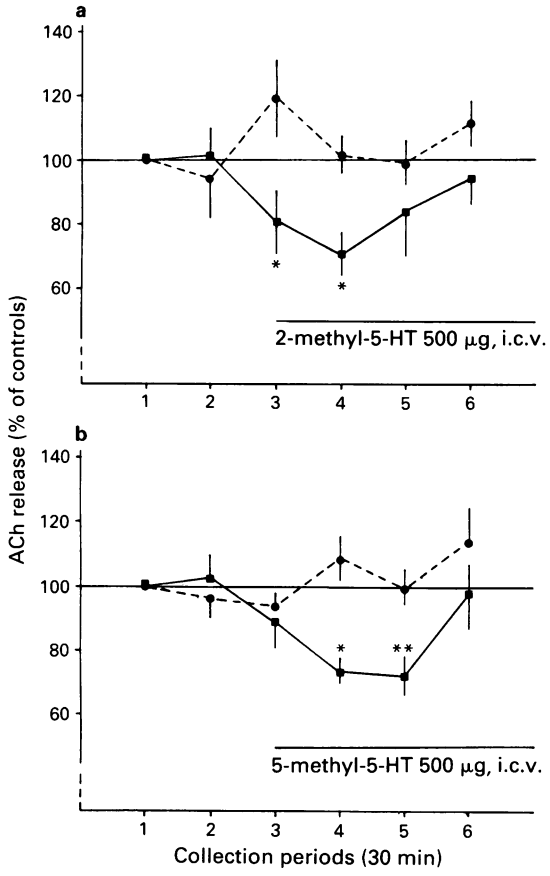

Figure 1 (a) Effect of 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) 500 μg i.c.v. (■, $n = 5$) and antagonism by MDL 72222, 1 mg kg^{-1} s.c. (●, $n = 5$) on acetylcholine (ACh) release from the cerebral cortex of control guinea-pigs. (b) Effect of 2-methyl-5-HT, 500 μg i.c.v. (■, $n = 5$) and antagonism by MDL 72222, 1 mg kg^{-1} s.c. (●, $n = 5$) on ACh release from the cerebral cortex of chronically (14 days) chlorimipramine (Cl-Imip)-treated guinea-pigs. Abscissa scales: collection periods (30 min); ordinate scales: percentage changes in ACh release. $*P < 0.05$; $**P < 0.01$ significantly different from the corresponding control, Student's t test for paired data. Control ACh release in chronically Cl-Imip-treated guinea-pigs was 127 ± 29 pmol cm^{-2} 30 min^{-1} and was not different from control guinea-pigs.

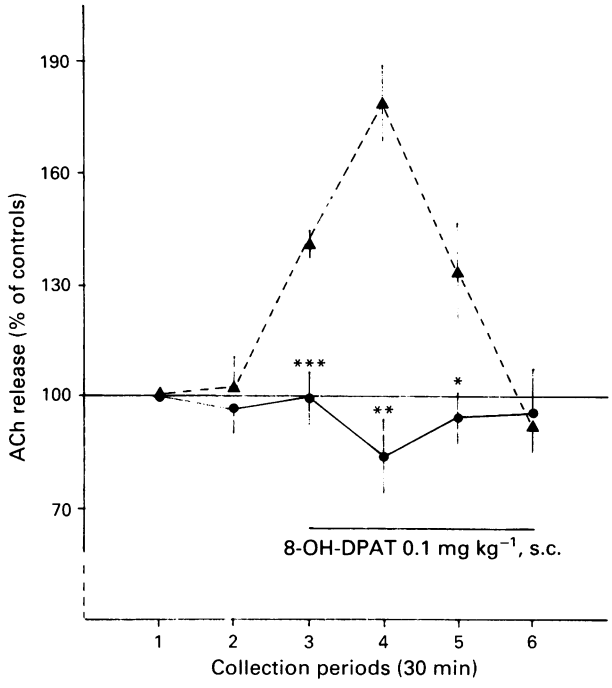

Figure 2 Lack of effect of 8-hydroxy-2-(di-n-propylaminotetralin) (8-OH-DPAT) 0.1 mg kg^{-1} s.c. on acetylcholine (ACh) release from the cerebral cortex of chronically chlorimipramine (Cl-Imip)-treated guinea pigs (●, $n = 6$). For comparison, the effect of 8-OH-DPAT in control animals (▲, $n = 8$) is shown. Abscissa scale: collection periods (30 min); ordinate scale: percentage changes in ACh release. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ significantly different from control animals, Student's t test for non paired data. Control ACh release in chronically Cl-Imip-treated guinea-pigs was 101 ± 18 pmol cm^{-2} 30 min^{-1} and was not significantly different from control guinea-pigs.

A rapid desensitization of 5-HT$_{1A}$ receptors following acute Cl-Imip administration is unlikely, because MDL 72222 restored the 8-OH-DPAT effect, indicating that the reduction was due to an opposing inhibitory effect of acute Cl-Imip, probably via 5-HT$_3$ receptors.

When Cl-Imip was acutely administered before 2-methyl-5-HT, the inhibitory effect of the latter drug on ACh release was not potentiated, suggesting a ceiling effect by 2-methyl-5-HT, 500 μg i.c.v. MDL 72222 was able to antagonize the inhibitory effect of the combination of Cl-Imip and 2-methyl-5-HT, suggesting an involvement of 5-HT$_3$ receptors.

In chronically Cl-Imip-treated guinea-pigs, 8-OH-DPAT was no longer able to produce its facilitatory effect on ACh release. The mechanism underlying this phenomenon was not the same as in acutely-treated animals, because the experiments were performed 24 h after the last Cl-Imip injection, a time compatible with the elimination half-life of the drug (Rudorfer & Potter, 1987). Moreover, a single injection of Cl-

Table 3 Effect of 2-methyl-5-hydroxytryptamine (2-methyl-5-HT, 500 μg i.c.v.) on the release of acetylcholine (ACh) from the cerebral cortex of freely moving guinea-pigs pretreated with saline, chlorimipramine (Cl-Imip, 10 mg kg^{-1} s.c.) or Cl-Imip plus MDL 72222 (1 mg kg^{-1} s.c.)

			ACh release			
		Before 2-methyl-5-HT	*After 2-methyl-5-HT*			
Pretreatment	*No expts.*	(control)	30 min	60 min	90 min	120 min
Saline[a]	5	123 ± 12	104 ± 13* (81)	83.4 ± 10* (71)	102 ± 16 (84)	110 ± 10 (94)
Cl-Imip[a]	4	109 ± 9.1	78.3 ± 11* (78)	67.9 ± 4.9* (70)	73.2 ± 7.1* (75)	89.2 ± 17 (88)
Cl-Imip[b]	3	122 ± 13	98 ± 11* (81)	109 ± 12 (88)	126 ± 14 (103)	126 ± 16 (103)
Cl-Imip[a] + MDL 72222	4	120 ± 18	122 ± 18 (102)	105 ± 12 (92)	119 ± 20 (101)	108 ± 19 (91)

[a] 30 min before 2-methyl-5-HT, [b] 24 h before 2-methyl-5-HT.
Values are pmol ACh cm^{-2} 30 min^{-1} ± s.e.mean; in parentheses the percentage of pretreatment values. $*P < 0.05$ significantly different from corresponding controls, Student's t test for paired data.

Imip, 24 h before the experiment, did not modify the 8-OH-DPAT effect. However, the possibility of accumulation of the drug, after chronic treatment, needs to be considered. Our data, although indirect, are against this possibility: basal ACh release 24 h after the last dose of Cl-Imip did not differ from normal values, and Cl-Imip still reduced ACh release, indicating that the acute inhibitory effect of the drug was extinguished, and that tolerance to this effect did not develop. Thus, it is likely that adaptive changes in 5-HT_{1A} receptors account for the lack of effect of 8-OH-DPAT in chronically Cl-Imip-treated guinea-pigs, in agreement with previous reports based on behavioural experiments (Goodwin *et al.*, 1985).

In contrast to 5-HT_{1A}-mediated effects, 5-HT_3 receptor-mediated inhibition did not seem to be affected by chronic Cl-Imip administration. In fact, the 5-HT_3 agonist, 2-methyl-5-HT, maintained its inhibitory, MDL 72222-sensitive effect in chronically treated guinea-pigs. Moreover, Cl-Imip, acutely administered to chronically treated animals, maintained its inhibitory effect as well.

The experimental approach used here does not shed light on any possible changes induced by Cl-Imip on other 5-HT receptor subtypes controlling ACh release. In fact, 5-HT_2 receptors are not involved in the control of cortical ACh release (Bianchi *et al.*, 1990) although they mediate a facilitatory effect on the basal ACh release from guinea-pig caudate nucleus slices (Bianchi *et al.*, 1989). The latter effect was prevented by chronic Cl-Imip treatment (Siniscalchi *et al.*, 1990b,c), supporting a down-regulation of 5-HT_2 receptors, in agreement with previous reports, based on binding studies (Peroutka & Snyder, 1980).

In conclusion, these results suggest that cortical ACh release is modulated in opposing ways by 5-HT_{1A} and 5-HT_3 receptor activation. In addition, it has been shown that chronic Cl-Imip treatment reduces the facilitatory response mediated by 5-HT_{1A} autoreceptors, while the inhibitory responses mediated by 5-HT_3 receptors are maintained. To our knowledge, modifications in 5-HT_3 functions or binding sites, following long-term antidepressant treatment, have not thus far been investigated. The differences in the adaptive changes shown by the various receptor classes may play a role in the antidepressant action of Cl-Imip.

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