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Displacement of striatal 5-hydroxytryptamine by dopamine released from endogenous stores

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Combined treatment with amfonelic acid and neuroleptics caused a considerable loss of striatal, but not cortical 5-hydroxytryptamine (5-HT) in rats. This could be prevented by pretreatment with the specific 5-HT uptake inhibitor, citalopram. The results are best explained by the assumption that the combined treatment with amfonelic acid and neuroleptic had massively released dopamine into the synaptic cleft, and a part of this had been taken up into the 5-HT neurons by the 5-HT uptake mechanism and displaced the indoleamine.

Amfonelic acid (AFA) is a non-amphetamine psychotogenic stimulant. Its biochemical actions on cerebral dopamine (DA) metabolism and behavioural effects are thought to result from its mobilization of the intraneuronal storage pool of DA which is probably related to its inhibition of reuptake (Shore et al 1979).

When classical neuroleptic drugs are administered to rats pretreated with AFA in doses sufficient to block its induced hyperactivity, their effect on the striatal concentrations of the DA metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) is greatly potentiated, and the concentration of the parent amine is markedly lowered (Shore 1976; McMillen 1981). On the other hand, atypical neuroleptics (in doses causing increases of deaminated DA metabolites similar to classical neuroleptics given alone) do not block the hyperactivity elicited by AFA, and their effects on HVA and DOPAC are not changed or even reduced by it (McMillen 1981; Waldmeier et al 1984). Nevertheless, the striatal concentration of DA is also reduced by such a combined treatment (Waldmeier et al 1984).

In some of the experiments of Waldmeier et al (1984), the striatal concentration of 5-HT was also measured and found to be reduced by the combination of AFA with neuroleptics, concomitantly with that of DA (unpublished). This unexpected phenomenon was subsequently studied in more detail, and these results are reported here.

Materials and methods

Haloperidol (Cilag AG, Schaffhausen, Switzerland) and perphenazine (Schering Corp., Kenilworth, NJ, USA) were obtained as injectable solutions. Amfonelic acid was kindly provided by Winthrop Labs, New York, NY, USA, clozapine 2HCl × H_2O and perlapine by Wander AG, Berne, Switzerland; metoclopramide HCl × H_2O by Kali-Chemie Pharma GmbH, Hannover, FRG; and spiperone by Janssen Pharmaceutica, Beerse, Belgium. Citalopram oxalate was synthesized in our Chemical Department by Dr R. Paioni.

Female Tif:RAlf (SPF) rats (Tierfarm Sisseln, Switzerland), 160–220 g received 2·5 mg kg^{-1} s.c. amfonelic acid 5 min before the test compounds and were decapitated 90 min later. This schedule has been used in the previous experiments on the interaction of neuroleptics and AFA with respect to the dopaminergic system. Preliminary experiments, not reported here, have shown that it is also suitable for the study of the interaction in terms of effects on 5-HT. In one experiment, the animals were also treated with the 5-HT uptake inhibitor, citalopram (30 mg kg^{-1} p.o. 25 min before AFA). Striata (in one experiment also cerebral cortices) were dissected from the brain and stored frozen at −20 °C until analysed. DA and 5-HT were determined by HPLC with amperometric detection as described previously (Waldmeier et al 1983).

The results presented in Table 1 demonstrate that in the animals which received both AFA and a test drug, striatal 5-HT was depleted to a similar extent as DA. The decreases of both amines were 30–60% of the control levels, which must be considered as strong effects. None of the neuroleptics or AFA decreased 5-HT or DA concentrations significantly when given alone.

The assumption was obvious that the excessive release of striatal DA was directly responsible for the 5-HT depletion, DA being taken up into the 5-HT stores by the 5-HT uptake mechanism and displacing the indoleamine. To check this, animals were pretreated with citalopram before they received AFA and haloperidol as an example of a neuroleptic agent. Citalopram is a highly specific inhibitor of 5-HT uptake, virtually devoid of effects on noradrenaline or DA uptake, and does not interact with adrenergic, cholinergic, dopaminergic, 5-HT or histamine receptors (Hyttel 1982). The results of this experiment are shown in Table 2. Again, a large decrease of the striatal DA concentration was accompanied by a similar decrease of that of 5-HT in the group receiving both haloperidol and AFA; no such effects occurred in the groups receiving either treatment alone, or citalopram, or the combinations of citalopram with AFA or haloperidol. On the other hand, pretreatment with citalopram prevented the decrease of striatal 5-HT caused by AFA + haloperidol, but not that of DA. In the cerebral cortex, which contains 5-HT in a concentration similar to that in the striatum, but only little DA, the combination of AFA

Table 1. Effect of combinations of AFA with several DA antagonists on striatal DA and 5-HT concentrations.

Treatment	Dose mg kg^{-1}	No AFA DA ng g^{-1}	No AFA 5-HT ng g^{-1}	With AFA DA ng g^{-1}	With AFA 5-HT ng g^{-1}
Controls	—	7813 ± 238 100%	756 ± 40 100%	7739 ± 468 99%	736 ± 39 97%
Spiperone	0·3 i.p.	6798 ± 196 87%	799 ± 47 106%	4584 ± 274[a] 59%	465 ± 15[a] 62%
Perlapine	20 p.o.	6869 ± 395 88%	640 ± 35 85%	4528 ± 486[b] 58%	398 ± 36[b] 53%
Metoclopramide	10 i.p.	6428 ± 135 82%	675 ± 57 89%	3840 ± 218[a] 49%	402 ± 19[b] 53%
Perphenazine	10 p.o.	7296 ± 277 93%	685 ± 29 91%	4006 ± 234[a] 51%	315 ± 64[a] 42%
Controls	—	11522 ± 486 100%	747 ± 10 100%	11389 ± 187 100%	686 ± 58 92%
Haloperidol	1 p.o.	10179 ± 208 89%	633 ± 34 85%	4661 ± 168[a] 41%	319 ± 22[a] 43%
Clozapine	100 p.o.	10641 ± 611 93%	647 ± 19 87%	4649 ± 419[a] 41%	477 ± 15[a] 64%

Groups of 4–6 rats were treated with amfonelic acid (AFA; 2·5 mg kg^{-1} s.c.) 5 min before the administration of the DA antagonists; 90 min later, the animals were decapitated and striatal DA and 5-HT concentrations determined. Data are means ± s.e.m.

[a] $P < 0{\cdot}001$, [b] $P < 0{\cdot}01$ vs DA antagonist given alone (Student's t-test).

and haloperidol did not reduce the levels of 5-HT.

In view of the specificity of citalopram, its preventive effect on the depletion of 5-HT induced by the combination of AFA and haloperidol can be assumed to be directly related to its 5-HT uptake inhibitory properties. An indirect effect via an influence on 5-HT synthesis is most unlikely; like all 5-HT uptake inhibitors, citalopram decreases 5-HT synthesis (Maitre et al 1982). However, to produce the observed effect by acting on 5-HT synthesis, an increase would be required. Most likely, the excessive amounts of DA released from dopaminergic nerve endings in the striatum by the combination of AFA and DA antagonists lead to high concentrations of DA in the immediate vicinity of the 5-HT nerve endings, much higher probably than those of 5-HT (the rat striatum contains about 10 times more DA than 5-HT). Thus, in spite of a lower affinity for DA than for 5-HT, the neuronal 5-HT uptake system is loaded with DA and transports it as a false substrate inside the 5-HT neurons, where it displaces 5-HT from its storage sites. Since citalopram inhibits the 5-HT uptake system, it prevents the access of the false substrate, DA, to the 5-HT neuron.

The displacement of striatal 5-HT by DA seems to occur to a marked extent under these experimental conditions, i.e. after massive release of DA. It might be expected, however, that this also occurs to a minor extent when DA release is enhanced to a lesser degree, e.g. after treatment with a DA antagonist, or a DA-releasing or uptake-inhibiting agent given alone. In this context, it is of interest that butyrophenones have been reported to significantly lower striatal 5-HT levels (Grabowska 1976); in our hands, a tendency in this direction was observed with most DA antagonists, which, however, was usually not statistically significant. It is not clear what the consequences of a displacement of striatal 5-HT by DA released by drugs would be. It might be anticipated that DA which has entered the 5-HT stores is co-released with 5-HT and reaches receptors it does not normally reach, or that 5-HT displaced by DA alters 5-HT transmission (if it gets outside the neuron intact), for instance. In any case, it is of interest that, in the intact animal, it is possible in a particular experimental situation that a transmitter enters the storage compartments of another transmitter.

Table 2. Prevention by citalopram of the reduction of the striatal 5-HT concentration by the combination of haloperidol and AFA.

Treatment	Dose mg kg^{-1}	Striatum DA ng g^{-1}	Striatum 5-HT ng g^{-1}	Cortex 5-HT ng g^{-1}
Controls	—	7694 ± 377 100%	781 ± 96 100%	407 ± 26 100%
Citalopram	30 p.o.	8149 ± 266 106%	808 ± 41 103%	449 ± 26 110%
AFA	2·5 s.c.	7810 ± 506 102%	698 ± 12 89%	417 ± 22 102%
Haloperidol	1 p.o.	6993 ± 326 91%	630 ± 24 81%	320 ± 19 79%
Citalopram + AFA	30 p.o. 2·5 s.c.	7260 ± 597 94%	823 ± 39 105%	448 ± 21 110%
Citalopram + haloperidol	30 p.o. 1 p.o.	7143 ± 402 93%	845 ± 38 108%	446 ± 24 110%
Haloperidol + AFA	1 p.o. 2·5 s.c.	4701 ± 199[a] 61%	413 ± 26[a] 53%	354 ± 34 87%
Citalopram + AFA + Haloperidol	30 p.o. 2·5 s.c. 1 p.o.	4751 ± 383[a] 62%	793 ± 22[b] 102%	410 ± 21 101%

Groups of 5 rats were treated orally with citalopram 25 min before the subcutaneous injection of AFA; 5 min after the latter, haloperidol was given orally. Ninety min later, the animals were decapitated. Data are given as means ± s.e.m.

[a] $P < 0{\cdot}01$ vs controls (Dunnett's test), [b] $P < 0{\cdot}001$ vs haloperidol + AFA (Student's t-test).

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Muscarinic cholinergic receptors in rabbit retina

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Binding sites with high affinity and specificity for [^{3}H]-quinuclidinyl benzilate are present in rabbit retinal homogenates. Only one set of binding sites was detected with an apparent dissociation constant of $2{\cdot}13 \times 10^{-10}$ M and a density of 59·2 fmol mg^{-1} of protein. The pharmacological characteristics of specific binding were similar to those in the goldfish, chicken and cow.

Acetylcholine (ACh) is believed to be a neurotransmitter substance in most, if not all, vertebrate retinas (for review see Neal 1983). The evidence is particularly strong in the rabbit retina, where two sub-populations of amacrine cells are believed to be cholinergic (Masland & Mills 1979). Electrophysiological, biochemical and autoradiographical experiments have emphasized the importance of nicotinic cholinergic receptors in the rabbit retina, but there is little information on the presence of muscarinic receptors in this species. For this reason, we have examined the muscarinic receptors in the rabbit retina using [^{3}H]quinuclidinyl benzilate (QNB).

Methods

Rabbit retinas were obtained from Buxted Olac Rabbits, Sussex, and were frozen intact immediately after dissection. Approximately 1 g of retinal tissue was homogenized in 15 ml of 50 mM Na-K phosphate buffer (pH 7·4) and then dispersed using a Polytron homogenizer (setting 6, 30 s). The homogenate was centrifuged at 27 000*g* for 10 min, resuspended and rehomogenized in buffer. It was then centrifuged at 49 000*g* for 10 min, resuspended and rehomogenized in buffer. Protein determinations were performed as described by Lowry et al (1951).

To demonstrate the presence of muscarinic cholinergic receptors in the rabbit retina, DL-[3-^{3}H]quinuclidinyl benzilate ([^{3}H]QNB, 10 Ci mmol^{-1}, Amersham International) was used as described previously (Yamamura & Snyder 1974). [^{3}H]QNB was incubated with retinal tissue with 2 ml of 50 mM Na-K phosphate buffer (pH 7·4) for 60 min at 37 °C. Incubation was terminated by rapid filtration through Whatman GF/C filters under vacuum. The filters were washed 4 times with 5 ml of ice cold buffer and then transferred to scintillation vials. 4 ml ethoxyethanol and 10 ml butyl PBD (0·5%) in toluene were added for liquid scintillation counting.

A duplicate set of tubes was incubated with 1 μM atropine to measure non-specific binding which was routinely subtracted from total binding to give the specific binding. All determinations were in triplicate and were repeated at least 4 times.

Results

The specific binding of [^{3}H]QNB increased linearly with the amount of retinal tissue over the range 0·1–0·8 mg per 2 ml assay volume. Increasing the concentration of [^{3}H]QNB resulted in saturation of the specific binding sites in the homogenate (Fig. 1). The non-specific binding measured after incubation with atropine (1 μM) was less than 17% of the total (Fig. 1).

A Scatchard plot (Scatchard, 1949; Fig. 1) revealed a single high affinity binding site, with an apparent dissociation constant (K_D) of $2{\cdot}13 \times 10^{-10} \pm 0{\cdot}21 \times 10^{-10}$ M (n = 3), while the maximum number of binding sites (B_{max}) was 59·2 ± 2·8 fmol mg^{-1} protein (n = 3).

The pharmacological specificity of [^{3}H]QNB binding to rabbit retinal tissue was determined from inhibition curves of several cholinoceptor agonists and antagonists (Fig. 2A). Hill plots of the inhibition curves obtained with atropine, d-tubocurarine, pilocarpine and carbachol are illustrated in Fig. 2B. Both the antagonists and

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