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INTERACTIONS BETWEEN RESERPINE AND VARIOUS COMPOUNDS ON THE ACCUMULATION OF [^{14}C] 5-HYDROXYTRYPTAMINE AND [^{3}H]NORADRENALINE IN HOMOGENATES FROM RAT HYPOTHALAMUS

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Abstract—The effect of reserpine, 5 mg/kg i.p., on the potencies of various compounds in inhibiting the accumulation of [^{14}C]5-hydroxytryptamine (5-HT) and [^{3}H] (–)-noradrenaline (NA) in synaptosome-rich homogenates from rat hypothalamus was examined. It was found that some compounds, e.g. tryptamine, α-methyltryptamine, (+)-amphetamine and 4-chloroamphetamine, were considerably more potent in inhibiting the 5-HT accumulation in the reserpinized preparation than in the control preparation, whereas other compounds, e.g. chlorimipramine, zimelidine, norzimelidine, fluoxetine, alaproclate and desipramine, had the same activities in the two preparations. The inhibitory potencies of the same compounds on the [^{3}H] NA accumulation was, on the other hand, not influenced by reserpine. The results indicate that tryptamines and amphetamines inhibit the 5-HT accumulation by releasing 5-HT rather than inhibiting its uptake. The same conclusion cannot be drawn for the inhibitors of the NA accumulation.

The neuronal accumulation of the biogenic monoamines in brain slices or synaptosomes by various drugs can be inhibited either by preventing the active transport of the amine through the neurone membranes or by a simultaneous release of the amine transported into the nerve terminals [1, 2]. In order to classify a compound as an uptake inhibitor it is therefore necessary to demonstrate that it does not release the amine in the same concentration range as it inhibits the accumulation [2]. This examination can be performed in two separate experiments, one recording the inhibition of the amine accumulation and the other measuring the release of the amine from pre-incubated tissue preparations [2–4]. Ideally these two experiments should be performed under identical conditions [2], which, however, is difficult to achieve. Although this technique differentiates between uptake inhibition and release for compounds which are more active in the former respect, it does not provide information of how more potent a compound is as a releasing agent than as an uptake inhibitor. Hence a drug may inhibit the amine accumulation and release the amine at the same concentration, but pharmacologically act predominantly as an uptake inhibitor.

In recent studies it was observed that reserpine enhances the potencies of amphetamine derivatives, ephedrine and phenmetrazine in inhibiting the accumulation of [^{3}H]dopamine in homogenates from rat striatum [5–7] but does not influence the potencies of cocaine, methylphenidate, pipradrol, amfonelic acid and nomifensine [5–7]. These findings indicate that the compounds in the former group inhibit the dopamine accumulation by releasing dopamine, whereas the compounds in the latter group are true uptake inhibitors. Comparison of the inhibitory potencies in normal and reserpinized synaptosome-rich brain homogenates may therefore become a valuable method of differentiating between true uptake inhibitors and releasing agents. This technique also gives an indication of how more potent a compound is in releasing the amine than in inhibiting its uptake.

The present study examines if similar differentiation between uptake inhibitors and releasing agents can be shown in the accumulation of [^{14}C]5-hydroxytryptamine (5-HT) and [^{3}H]noradrenaline (NA) in homogenates from rat hypothalamus. The compounds tested are uptake inhibitors such as chlorimipramine [8], desipramine [8], zimelidine [9], norzimelidine [9], fluoxetine [10], 3,3-diphenylcyclopentylamine [11], and alaproclate [12] and releasing agents, such as phenethylamine, (+)-amphetamine [13], 4-chloroamphetamine [13], tryptamine and α-methyltryptamine (unpublished observations).

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 180–240 g were used. Hypothalamus was dissected out and the pooled hypothalami from 8 to 10 rats were homogenized in 15 vol. ice-chilled 0.25 M sucrose in all-glass Potter–Elvehjems homogenizers. The homogenates were centrifuged at 800 *g* for 10 min. The synaptosome-rich supernatant was used.

The simultaneous accumulation of [^{3}H]NA and [^{14}C]5-HT in the synaptosomes was determined as described previously [8]. The incubation mixture in PVC centrifuge tubes consisted of 100 μl of the homogenate, 5×10^{-8} M [^{3}H]NA, 5×10^{-8} M [^{14}C]5-HT, the test compound, 5×10^{-5} M pargyline, 1.1 mM ascorbic acid, 1.3×10^{-4} M EDTA disodium salt, 5.6 mM glucose in 1.85 ml of Krebs–Henseleit's buffer, pH 7.4. The mixture was pre-incubated for 5 min at 37° or 0° before the addition of the labelled amines. The incubation was continued for a further 4 min and the amine

accumulation was stopped by chilling the tubes in ice-water. The pellets collected by centrifugation at 15,000 *g* for 20 min were washed twice with 5 ml saline. The pellets were dissolved in 1.0 ml Soluene-350 (Packard) and 10 ml of scintillation liquid (Permablend III, Packard in toluene) was added. The active amine accumulation was calculated from the difference between the radioactivities in the pellets incubated at 37° and 0°. Duplicates of seven different concentrations of the test compound were recorded. Homogenates from control rats and reserpinized rats were always examined in the same experiment. IC_{50} values and 95 per cent confidence limits were calculated by linear regression analysis from the approximately linear part of the log dose–response curves.

Compounds. (−)-Noradrenaline-7 [^{3}H] (sp. act. 3.0 Ci/m-mole) was purchased from NEN GmbH, Frankfurt/Main, West Germany and 5-hydroxytryptamine (side-chain [2 ^{14}C]) creatinine sulphate (specific activity 54 mCi/m-mole) from the Radiochemical Centre, Amersham, England. 4-Chloroamphetamine hydrochloride was bought from Regis Chemical Co and α-methyltryptamine hydrochloride from Calbiochem. (+)-Amphetamine sulphate and methylphenidate hydrochloride were purchased from Apoteket Sländan, Stockholm, Sweden. Chlorimipramine hydrochloride and desipramine hydrochloride were kindly provided by Ciba-Geigy AG and fluoxetine hydrochloride by Eli Lilly Co. Phenethylamine was purchased from Aldrich Chemical Co. Zimelidine dihydrochloride, norzimelidine dihydrochloride (the monomethyl derivative of zimelidine), alaproclate hydrochloride (2-[4-chlorophenyl]-1,1-dimethyl 2-aminopropanoate hydrochloride) and 3,3-diphenylcyclopentylamine hydrochloride were synthesized in the Astra Research Laboratories.

RESULTS

Accumulation of [^{14}C]*5-HT.* The accumulation of [^{14}C]5-HT in the synaptosome-rich hypothalamic homogenate was significantly decreased by pre-treatment of the rats with reserpine, 5 mg/kg i.p. 18 hr before the experiments (Table 1). The accumulation was 59 per cent of that in the control preparation. Many of the compounds examined had similar inhibitory potencies in the reserpinized preparation and in the control homogenate (Fig. 1, Table 2). To this group belong the very potent uptake inhibitors chlorimipramine [8], norzimelidine [9] and fluoxetine [10]; the moderately active 3,3-diphenylcyclopentylamine [11], zimelidine [9], alaproclate [12] and desipramine [8]; and the very weak inhibitor tyramine. Some compounds were, on the other hand, twice or several-fold more active in the reserpinized homogenate than in the control preparation. Hence, α-methyltryptamine was 10 times more potent in the reserpinized homogenate. To this group belongs also 4-chloroamphetamine. (+)-Amphetamine, phenethylamine and methylphenidate had very low activity and (+)-amphetamine was twice as potent in the reserpinized tissue as in the normal preparation.

Table 1. Accumulation of [^{3}H](−)-noradrenaline (NA) and [^{14}C]5-hydroxytryptamine (5-HT) in synaptosome-rich homogenates of hypothalamus from non-treated and reserpinized rats *

Amine	Amine accumulation, pmoles/mg wet tissue/min ± S.E.M. Control	Reserpine	P†
[^{3}H]NA	0.068 ± 0.003	0.038 ± 0.002	< 0.001
[^{14}C]5-HT	0.270 ± 0.010	0.159 ± 0.008	< 0.001

* Reserpine, 5 mg/kg i.p., was injected 18 hr before the experiments. The active accumulation of the labelled amines (5×10^{-8} M) was determined as the difference in radioactivity in the synaptosomes taken up at 37° and 0° during 4 min incubation. Each value is the mean ± S.E.M. of 5 determinations.

† Student's *t* test.

Table 2. Effect of reserpine on the inhibition of the accumulation of [^{14}C]5-HT in synaptosome-rich rat hypothalamic homogenates *

Compound	IC_{50} μM (95 per cent confidence limits) Control	Reserpine	Control Reserpine
(+)-Amphetamine	12.4 (11.0–14.1)	6.0 (3.4–11.2)	2.1
4-Chloroamphetamine	0.32 (0.28–0.37)	0.077 (0.056–0.120)†	4.2
Phenethylamine	92 (40–154)	40 (21–144)	2.3
Methylphenidate	> 37	> 37	—
Tyramine	12.0 (9.3–16.0)	11.3 (10.4–12.3)	1.1
Tryptamine	0.27 (0.19–0.40)	0.058 (0.026–0.106)†	4.7
α-Methyltryptamine	0.19 (0.16–0.22)	0.018 (0.010–0.032)†	10.6
3,3-Diphenylcyclopentylamine	0.15 (0.12–0.19)	0.15 (0.10–0.25)	1.0
Chlorimipramine	0.011 (0.009–0.013)	0.013 (0.008–0.036)	0.8
Zimelidine	0.33 (0.28–0.41)	0.34 (0.25–0.52)	1.0
Norzimelidine	0.056 (0.046–0.070)	0.051 (0.035–0.081)	1.1
Fluoxetine	0.062 (0.049–0.082)	0.043 (0.038–0.049)	1.4
Alaproclate	0.37 (0.29–0.50)	0.28 (0.23–0.34)	1.3
Desipramine	0.68 (0.33–2.27)	0.71 (0.52–1.02)	1.0

* Cell-free homogenates (1:15) in 0.25 M sucrose from non-treated or reserpinized rats were pre-incubated with the inhibitor for 5 min and incubated with the labelled amines (5×10^{-8} M) for 4 min. The IC_{50} values and the 95 per cent confidence limits were determined by linear regression analysis of the approximately linear part of the log dose–response curves based on 7 different concentrations of the inhibitor.

† $P < 0.05$.

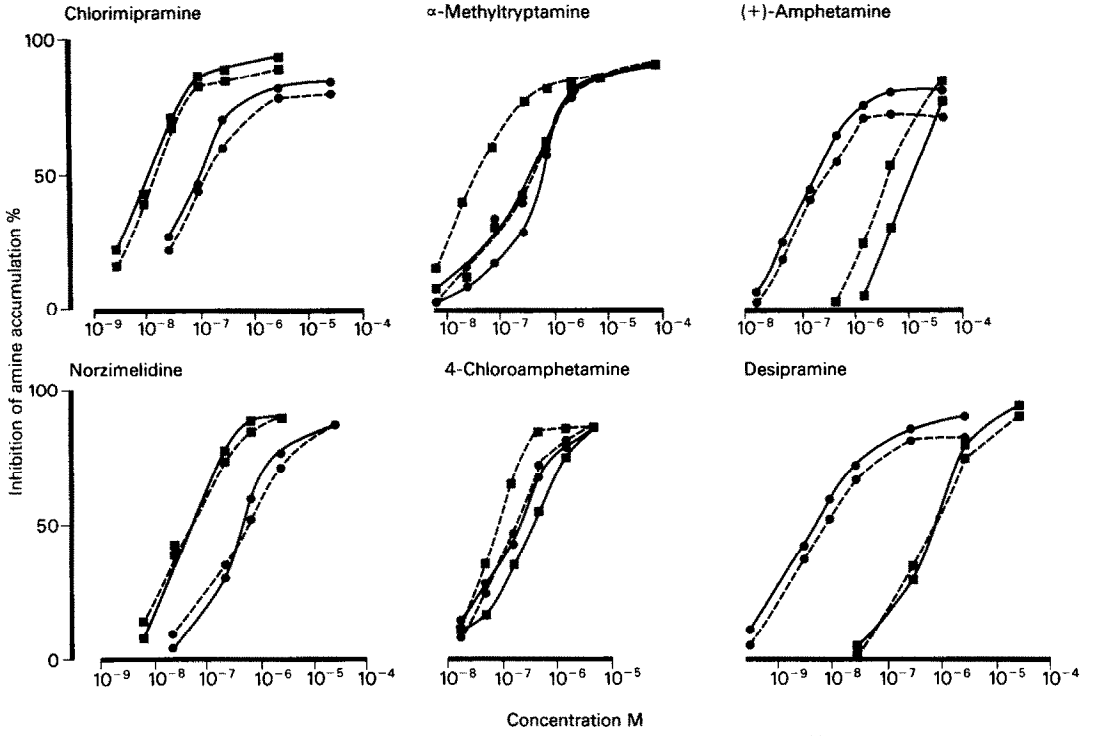

Fig. 1. Dose–response curves of the inhibition of the accumulation of [^{14}C]5-HT (■) and [^{3}H]NA (●) in synaptosome-rich hypothalamic homogenates from non-treated (whole lines) and reserpinized (broken lines) rats. Each point is the mean of duplicate determinations. See legend to Table 2 for the incubation conditions.

Accumulation of [^{3}H]*NA*. The accumulation of [^{3}H]NA in the reserpinized homogenate was about 56 per cent of that in the control preparation (Table 1). Except for α-methyltryptamine, which was most active in the reserpinized homogenate, all compounds examined had similar potencies in the two preparations (Fig. 1, Table 3). It can be noted that chlorimipramine, norzimelidine and fluoxetine were about 10 times and alaproclate 100 times less active in inhibiting the accumulation of NA than that of 5-HT, whereas desipramine was 100 times more active on the NA accumulation.

DISCUSSION

The results obtained show that the potencies of some compounds in inhibiting the [^{14}C]5-HT accumulation in the homogenate from rat hypothalamus were significantly enhanced by pretreatment of the rats with reserpine, whereas those of other compounds were not changed by reserpine. Hence, the accumulation of [^{14}C]5-HT behaves in this respect like that of [^{3}H]dopamine in rat striatal homogenates [5–7]. In both cases compounds which are known to be potent amine releasing agents were potentiated by reserpine.

Table 3. Effect of reserpine on the inhibition of the accumulation of [^{3}H](−)-noradrenaline in synaptosome-rich rat hypothalamic homogenates*

Compound	IC_{50} μM (95 per cent confidence limits) Control	Reserpine	Control Reserpine
(+)-Amphetamine	0.26 (0.20–0.37)	0.37 (0.24–0.66)	0.7
4-Chloroamphetamine	0.20 (0.15–0.28)	0.18 (0.13–0.20)	1.1
Phenethylamine	0.23 (0.20–0.26)	0.21 (0.16–0.29)	1.1
Methylphenidate	1.60 (1.18–2.38)	1.47 (1.07–2.22)	1.1
Tyramine	0.18 (0.13–0.26)	0.14 (0.05–0.64)	1.3
Tryptamine	0.77 (0.39–1.43)	0.82 (0.61–1.11)	0.9
α-Methyltryptamine	0.33 (0.26–0.44)	0.18 (0.14–0.25)†	1.8
3,3-Diphenylcyclopentylamine	0.40 (0.35–0.45)	0.56 (0.41–0.81)	0.7
Chlorimipramine	0.10 (0.073–0.15)	0.15 (0.12–0.20)	0.7
Zimelidine	8.2 (3.4–20.6)	15.2 (6.8–115)	0.5
Norzimelidine	0.56 (0.40–0.73)	0.68 (0.52–0.87)	0.8
Fluoxetine	0.76 (0.50–1.4)	0.82 (0.43–2.2)	0.9
Alaproclate	> 34	> 34	—
Desipramine	0.0056 (0.0046–0.0066)	0.0083 (0.0063–0.0116)	0.7

* Determined simultaneously with that of [^{14}C]5-HT (see legend to Table 2).
† $P < 0.05$.

The potencies of the inhibitors of the amine transport across the neuronal membrane were, on the other hand, unaffected or only slightly enhanced by reserpine. A plausible conclusion is, therefore, that compounds like tryptamine, α-methyltryptamine, (+)-amphetamine and 4-chloroamphetamine are more potent in releasing 5-HT than in inhibiting its uptake. Drugs such as chlorimipramine, norzimelidine, zimelidine, fluoxetine, alaproclate, 3,3-diphenylcyclopentylamine and desipramine are, on the other hand, more potent in inhibiting the uptake than in releasing 5-HT.

If it is assumed that reserpine enhances the inhibitory potencies of the releasing agents by blocking the vesicular 5-HT binding, the increased importance of some extravesicular binding sites of 5-HT, to which these agents also have high affinity, may explain the potentiation. In the reserpinized tissue the occupancy of these sites by the releasing agents should give a marked decrease in the [^{14}C]5-HT accumulation, since the rate of the outward transport (release) of [^{14}C]5-HT taken up is accelerated. In fact release of 5-HT from extravesicular sites by 4-chloroamphetamine is indicated by the observation that behaviour effects, e.g. head-twitches and tremor, which are most likely due to central 5-HT receptor activation, are produced by this agent in reserpinized mice [14].

According to the hypothesis proposed the inhibitory potencies of the releasing agents in the reserpinized preparation reflect their affinities to some extravesicular, but intraneuronal, 5-HT binding sites. The question then arises as to whether the inhibitory potencies of these compounds in the control preparation (1) measure the inhibition of the membrane transport, (2) are due to the same release mechanism, but higher concentration of the compounds are necessary because of the intact vesicular binding mechanism, or (3) are a mixed effect of these two actions. In addition release of 5-HT from vesicular binding sites cannot be excluded in this preparation. The experiments performed in this study do not give any answer to this question. However, it can be concluded that the inhibitory potencies on the membrane 5-HT transport cannot be higher than that obtained in the control preparation. Thus, the ratio between releasing potency and uptake inhibitory activity is at least as large as indicated in Table 2.

The inhibition of the [^{3}H]NA accumulation in hypothalamus differs from that of dopamine and 5-HT accumulation and also from that of NA in peripheral noradrenergic nerve terminals [15], since reserpine did not enhance the potency of the compounds examined (with the possible exception of α-methyltryptamine). This finding should accordingly indicate that these compounds are more potent as inhibitors of the NA uptake than in releasing NA from central noradrenergic neurones. The observations that (+)-amphetamine is a rather weak releasing agent of [^{3}H]NA from brain slices [16, 17], and that this effect is not enhanced by reserpine [17] are also in favour of this interpretation. However, a minor fraction (~20 per cent) of the [^{3}H]NA accumulated in cerebral cortical slices (and probably not accumulated in dopaminergic neurones) can be released by (+)-amphetamine, which had a significant effect at rather low concentrations (1×10^{-7} M) [17]. Reserpine increased the spontaneous release of [^{3}H]NA from cortical slices much more than that of [^{3}H]DA from striatal slices [17]. A possible explanation of these findings is that the extravesicular binding sites in central noradrenergic neurones are less numerous than those in dopaminergic and peripheral noradrenergic neurones.

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