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Is There a Functional Linkage Between Neurotransmitter Uptake Mechanisms and Presynaptic Receptors?[1]

MAURIZIO RAITERI, GIAMBATTISTA BONANNO, MARIO MARCHI and GUIDO MAURA
Istituto di Farmacologia e Farmacognosia, Università di Genova, Viale Cembrano 4, 16148 Genova, Italy
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ABSTRACT

The possibility of interaction between neurotransmitter uptake mechanisms and presynaptic receptors regulating transmitter release was investigated using rat brain synaptosomes in superfusion. Various conditions were considered including: 1) absence of substrate for the uptake with uptake potentially operative; 2) absence of substrate and presence of uptake inhibitors; and 3) uptake activated by added substrate, with or without uptake inhibitors. The release of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) evoked by 15 mM KCl from cerebral cortex synaptosomes was inhibited by lysergic acid diethylamide. The 5-HT uptake inhibitors citalopram and chlorimipramine did not affect the inhibitory action of lysergic acid diethylamide. Clonidine decreased both the K^+-evoked release of [^{3}H]norepinephrine and that of [^{3}H]-5-HT in cortical synaptosomes through the activation of presynaptic *alpha*-2 adrenoceptors. In superfusion conditions, the action of clonidine on [^{3}H]norepinephrine release was not antagonized by the norepinephrine uptake inhibitors desipramine or cocaine; similarly, the inhibition of [^{3}H]-5-HT release was unaffected when 5-HT uptake was blocked. The K^+-evoked release of [^{3}H]dopamine from striatal nerve terminals was potentiated by acetylcholine (ACh) through the activation of muscarinic presynaptic receptors. The action of ACh was not modified by the presence of nomifensine, a dopamine uptake inhibitor. Finally, in superfused cortical synaptosomes, the block of the high-affinity uptake of choline by hemicholinium-3 had no effect on the muscarinic autoreceptor-mediated inhibition of [^{3}H]ACh release by ACh. Altogether the present results do not support the previously proposed idea that in nerve terminals a functional coupling may exist between uptake mechanisms and presynaptic receptors.

It is well established that nerve terminals in the central nervous system are endowed with presynaptic receptors that mediate regulation of neurotransmitter release by agents present in the extracellular fluid. The release of a given transmitter can be regulated by the transmitter itself, through the activation of presynaptic autoreceptors or by other transmitters or modulators through the activation of presynaptic heteroreceptors (for reviews see Langer, 1981; Starke, 1981, Raiteri *et al.*, 1984).

Central nerve terminals are also endowed with membrane carrier systems involved in the reuptake of the transmitters released previously into the synaptic cleft (for reviews see Iversen, 1971; Levi and Raiteri, 1976).

Experiments performed with brain slices have shown that auto- and heteromodulations of transmitter release by presynaptic receptor agonists can be prevented when inhibitors of the reuptake of the released transmitter are present (Starke and Montel, 1973; Pelayo *et al.*; 1980; Galzin *et al.*, 1982; Langer and Moret, 1982; Reichenbacher *et al.*, 1982; Cubeddu *et al.*, 1983; Göthert *et al.*, 1983).

The interaction between clonidine and NE uptake blockers was observed first (Starke and Montel, 1973). Although the observation has been confirmed several times, there is no agreement on its interpretation. According to Starke and collaborators (Starke and Montel, 1973; Reichenbacher *et al.*, 1982), the action of clonidine as an inhibitor of the stimulated [^{3}H]NE release is counteracted by the NE released during stimulation which, in the presence of a NE reuptake inhibitor, accumulates in the vicinity (biophase) of the presynaptic *alpha*-2 autoreceptors. As an alternative explanation, Pelayo *et al.* (1980) proposed that the inhibitors of NE uptake may interact with an "imidazoline recognition site" localized on the *alpha*-2 autoreceptors, thus preventing their activation by clonidine or other *alpha*- agonist imidazolines.

Subsequently, when the interactions between presynaptic receptor agonists and neurotransmitter uptake inhibitors appeared to represent a generalized phenomenon, a new hypothesis was proposed that implies the existence in nerve endings of a functional coupling between mechanisms of transmitter uptake and presynaptic receptor mechanisms (Galzin *et al.*, 1982; Langer and Moret, 1982; Göthert *et al.*, 1983). Some authors, however, still believe that the accumulation of the endogenous transmitter released in the receptor biophase plays

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ABBREVIATIONS: NE, norepinephrine; 5-HT, 5-hydroxytryptamine; DA, dopamine; LSD, lysergic acid diethylamide, ACh, acetylcholine.

the critical role (Reichenbacher *et al.*, 1982; Cubeddu *et al.*, 1983).

Inasmuch as several inhibitors of neuronal transmitter uptake are used as antidepressants, the possibility for these drugs to modulate presynaptic receptor function would be of pharmacological and therapeutic relevance. In the present study, the problem of the interactions between uptake mechanisms and presynaptic receptors are re-evaluated using isolated nerve endings in superfusion (Raiteri *et al.*, 1974). As previously shown (see Raiteri and Levi, 1978; Raiteri *et al.*, 1984) in these experimental conditions, the released transmitters are rapidly removed by the superfusion fluid before they can interact with reuptake sites and presynaptic receptors. It is therefore possible to study the function of presynaptic receptors and their possible interactions with the uptake mechanisms by exposing the nerve endings to controlled amounts of presynaptic receptor agonists, under the following conditions: 1) when reuptake does not operate, but its mechanisms are potentially effective; 2) when uptake is rendered operative by adding controlled amounts of substrate to the superfusion medium; and 3) when the nerve endings are exposed to selective uptake inhibitors.

Materials and Methods

Crude synaptosomes were prepared essentially according to the method of Gray and Whittaker (1962) with some modifications. Briefly, the whole cerebral cortex or the corpus striatum of adult male Sprague-Dawley rats (200–250 g) were homogenized in 40 volumes of 0.32 M sucrose. The homogenate was centrifuged (5 min, 1000 × *g*) to remove nuclei and debris and synaptosomes were isolated from the supernatant by centrifugation at 12,000 × *g* for 20 min. The synaptosomal pellet was then resuspended (at a protein concentration of 0.5–0.6 mg/ml) in a physiological salt solution having the following composition (millimolar): NaCl, 125; KCl, 3; $MgSO_4$, 1.2; $CaCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 20; and glucose, 10 (aeration with 95% O_2 and 5% CO_2 at 37°C), pH 7.2 to 7.4. Protein was measured by the method of Bradford (1976).

Synaptosomes were preincubated 10 min at 37°C in a rotary waterbath and were subsequently labeled with [^{3}H]-5-HT (10 min; final concentration, 0.03 μM), [^{3}H]NE (10 min; final concentration, 0.04 μM), [^{3}H]DA (10 min; final concentration, 0.01 μM) or with [^{3}H]choline (4 min; final concentration, 0.1 μM).

After labeling, aliquots of the synaptosomal suspensions were distributed on 0.65-μm Millipore filters (0.3–0.5 mg of protein per filter), placed at the bottom of as many as 20 parallel superfusion chambers thermostated at 37°C (Raiteri *et al.*, 1974) and superfusion was then started, at a rate of 0.6 ml/min, with standard medium continuously aerated with 5% CO_2 in O_2. Whenever used, uptake inhibitors (citalopram, chlorimipramine, desipramine, cocaine, nomifensine and hemicholinium-3) and receptor antagonists (methiothepin, yohimbine and atropine) were present from the beginning of superfusion; the agonists LSD and clonidine were added at 10 min, ACh at 15 min. In the experiments aimed to study presynaptic receptor function in conditions in which the uptake systems are operative, the uptake substrates were added at the beginning or at 18 min of superfusion, as indicated in table 1. Depolarization was obtained by exposure to 15 mM KCl (substituting an equimolar concentration of NaCl) at 18 min of superfusion. Superfusate samples were collected every minute and the radioactivity present in the samples and in the synaptosomes at the end of the superfusion was determined by liquid scintillation counting. The radioactivity found in each superfusate sample was calculated as a percentage of the total tritium recovered (samples plus superfused synaptosomes).

In order to evaluate the K^+-evoked release, the fractional rate of the basal efflux (average of the two 1-min superfusate samples immediately preceding the onset of K^+-stimulation) was subtracted from the fractional rate corresponding to the maximal release observed during high-K^+ exposure. The results in the figures are therefore expressed as percentage of the release evoked by high-K^+ in the absence of drugs. Under the present experimental conditions, the radioactivity released by K^+-stimulation from synaptosomes prelabeled with [^{3}H]-5-HT, [^{3}H]NE, [^{3}H]DA or [^{3}H]choline consisted, for the major part of the unmetabolized transmitters, as shown previously by chromatographic and radioenzymatic analysis (Raiteri *et al.*, 1982; Maura *et al.*, 1982a, 1982b, Marchi *et al.*, 1983).

Experiments of uptake in incubation and in superfusion conditions were performed as follows: the same amount of synaptosomes (0.2–0.3 mg of protein) were either incubated or superfused for 5 min at 37°C with a medium containing 0.01 μM [^{3}H]-5-HT or with 0.1 μM [^{3}H]choline. Blanks were obtained incubating or superfusing synaptosomes at 0–2°C The filters holding the superfused synaptosomes and those on which the incubated synaptosomes were collected were then washed with standard medium and the radioactivity was counted in a liquid scintillation system.

Chemicals. L-[7,8-^{3}H]NE (specific activity, 32 Ci/mmol) and [7,8-^{3}H]DA (specific activity, 46 Ci/mmol) were obtained from the Radiochemical Centre (Amersham, England); [1,2-^{3}H(N)]-5-HT creatinine sulfate (specific activity, 30.1 Ci/mmol) and [^{3}H]choline (specific activity, 70 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Yohimbine, ACh hydrochloride and atropine sulfate were purchased from Sigma Chemical Co. (St. Louis, MO), (−)-arterenol bitartrate hydrate and 5-HT creatinine sulfate from Calbiochem (Los An-

TABLE 1

Effect of neuronal uptake activation on presynaptic receptor function

Synaptosomes prelabeled with [^{3}H]NE, [^{3}H]choline or [^{3}H]-5-HT were superfused in the absence or in the presence of the corresponding substrates of the neuronal carrier (exogenous tyramine or 5-HT added at 18 min and exogenous choline at the begining of superfusion). During depolarization with 15 mM KCl the presynaptic inhibitory *alpha*-2 adrenoceptors present on NE and 5-HT terminals were then activated with clonidine and the presynaptic inhibitory muscarinic autoreceptors located on ACh terminals with exogenous ACh. When indicated selective inhibitors of neuronal uptake were added at 10 min of superfusion. Means ± S.E.M. of three experiments in quadruplicate are presented.

Treatment	% Inhibition of K^+-Evoked Release
[^{3}H]Norepinephrine Release	
Control (15 mM KCl)	
Clonidine, 0.5 μM	30.5 ± 3.55
Control with uptake substrate (15 mM KCl + 0.1 μM Tyramine)	
Clonidine, 0.5 μM	28.0 ± 2.38
Control with uptake substrate and uptake inhibitor (15 mM KCl + 0.1 μM Tyramine + 1 μM Desipramine)	
Clonidine, 0.1 μM	29.1 ± 3.25
[^{3}H]Acetylcholine Release	
Control (15 mM KCl)	
ACh, 5 μM	40.6 ± 4.0
Control with uptake substrate (15 mM KCl + 0.1 μM Choline)	
ACh, 5 μM	36.8 ± 4.2
Control with uptake substrate and uptake inhibitor (15 mM KCl + 0.1 μM Choline + 100 μM HC-3)	
Ach, 5 μM	35.1 ± 4.7
[^{3}H]5-Hydroxytryptamine Release	
Control (15 mM KCl)	
Clonidine, 0.3 μM	29.7 ± 1.9
Control with uptake substrate (15 mM KCl + 0.01 μM 5-HT)	
Clonidine, 0.3 μM	26.9 ± 1.5

geles, CA) and hemicholinium-3 from Aldrich-Europe (Beerse, Belgium). The following compounds were gifts: LSD (Sandoz, Basel, Switzerland), chlorimipramine and desipramine (Ciba Geigy, Basel, Switzerland), citalopram (Lundbeck, Copenhagen, Denmark), nomifensine (Hoechst, Frankfurt, FRG) and clonidine (Boehringer Ingelheim Int., FRG).

Results

Inhibition by LSD of the [^{3}H]-5-HT release elicited by 15 mM KCl in synaptosomes of rat cerebral cortex and study of the interaction between LSD and the 5-HT uptake inhibitors citalopram and chlorimipramine. Autoreceptors are present on central 5-HT terminals and their activation leads to a reduction of the evoked release of [^{3}H]-5-HT that can be reversed by methiothepin (Bourgoin *et al.*, 1977; Cerrito and Raiteri, 1979; Göthert and Weinheimer, 1979; Langer and Moret, 1982). Depolarization with high-K^+ increased the release of [^{3}H]-5-HT from cortical synaptosomes prelabeled with the radioactive amine. Under the experimental conditions used, 0.03 μM [^{3}H]-5-HT labeled preferentially the 5-HT nerve terminals, as nomifensine, a potent inhibitor of the DA and NE carriers (Schacht and Leven, 1984) did not decrease significantly the uptake of [^{3}H]-5-HT in cortical synaptosomes (control uptake: 0.44 ± 0.05 pmol/mg of protein per min; percentage of inhibition by 0.1 μM nomifensine: 3.5 ± 0.7; n = 5). The uptake of [^{3}H]-5-HT was, however, strongly inhibited by 0.1 μM citalopram (percentage of inhibition: 87.5 ± 5.1; n = 5). The K^+-evoked release of [^{3}H]-5-HT from prelabeled rat cortical synaptosomes was previously shown to be largely calcium-dependent (Maura *et al.*, 1982b). Exposure to LSD (0.003–0.3 μM) decreased the depolarization-evoked release of [^{3}H]-5-HT in a concentration-dependent manner (fig. 1). The action of LSD (0.03 μM) was antagonized by 0.3 μM methiothepin which, by itself, did not affect the K^+-evoked release. The inhibitors of neuronal 5-HT uptake, citalopram and chlorimipramine, at 0.3 μM, did not modify, by themselves, the K^+-evoked overflow of [^{3}H]-5-HT. These compounds did not affect significantly the inhibition of [^{3}H]-5-HT release by LSD, even when the ratio between uptake inhibitor and autoreceptor agonist was 100.

Inhibition by clonidine of the [^{3}H]-5-HT release elicited by 15 mM KCl in synaptosomes of the rat cerebral cortex and study of the interaction between clonidine and the 5-HT uptake inhibitor chlorimipramine. The evoked release of [^{3}H]-5-HT can be decreased through the activation of presynaptic heteroreceptors of the *alpha*-2 type (Göthert *et al.*, 1981; Frankhuyzen and Mulder, 1982; Maura *et al.*, 1982a; Raiteri *et al.*, 1983). Figure 2 shows that the K^+-evoked release of [^{3}H]-5-HT in superfused cortex synaptosomes was inhibited by clonidine (0.03–1 μM) in a concentration-dependent manner. The action of clonidine (0.3 μM) was counteracted by the *alpha*-2 adrenoceptor antagonist yohimbine (0.3 μM) which, by itself, did not modify the K^+-evoked release of [^{3}H]-5-HT. The presence of chlorimipramine in the superfusion fluid did not affect the inhibitory action of the *alpha*-2 agonist imidazoline. At the concentration used (0.3 μM), chlorimipramine did not modify, by itself, the K^+-evoked release.

Inhibition by clonidine of the [^{3}H]NE release elicited by 15 mM KCl in synaptosomes of the rat cerebral cortex and study of the interactions between clonidine and the NE uptake inhibitors desipramine and cocaine. Central NE terminals are endowed with autoreceptors that belong to the adrenergic *alpha*-2 type (Langer, 1981; Starke, 1981). Depolarization with high-K^+ stimulated the release of [^{3}H]NE from superfused cortical synaptosomes prelabeled with the radioactive amine. Under these experimental conditions that K^+-evoked release was found to be largely calcium-dependent (Raiteri *et al.*, 1979). Clonidine (0.003–1 μM) inhibited the K^+-evoked [^{3}H]NE release in a concentration-dependent way (fig. 3). The action of 1 μM clonidine was totally antagonized by 1 μM yohimbine which, by itself, did not modify the K^+-evoked [^{3}H]NE release. The inhibitory action of clonidine was not affected significantly when the NE uptake blockers desipramine or cocaine were present. The NE uptake inhibitors, at 1 μM, did not modify, by themselves, the K^+-evoked release of the catecholamine.

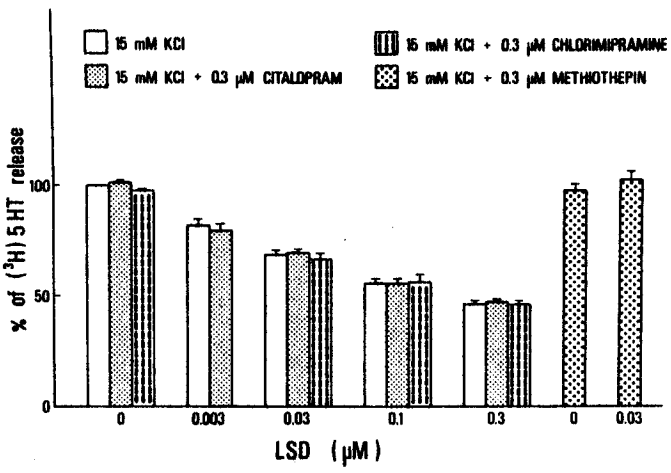

Fig. 1. Autoreceptor-mediated inhibition of [^{3}H]-5-HT release by LSD: effects of citalopram, chlorimipramine and methiothepin. Rat cerebral cortex synaptosomes were labeled with [^{3}H]-5-HT and depolarized in superfusion with 15 mM KCl as described under "Materials and Methods." The absolute value of fractional release evoked by 15 mM KCl (see "Materials and Methods") was 1.85 ± 0.08. The values displayed and means ± S.E.M. of four to six experiments in quadruplicate.

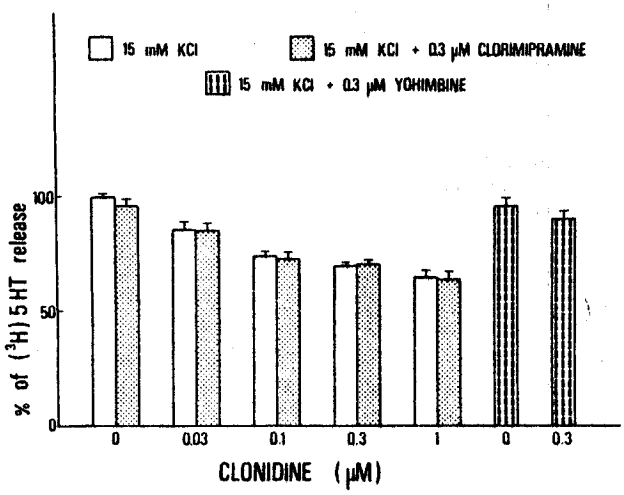

Fig. 2. *Alpha*-2 adrenoceptor-mediated inhibition of [^{3}H]-5-HT release by clonidine: effects of chlorimipramine and of yohimbine. Rat cerebral cortex synaptosomes were labeled with [^{3}H]-5-HT and depolarized in superfusion with 15 mM KCl as described under "Materials and Methods." The absolute value of fractional release evoked by high-K^+ (see "Materials and Methods") was 1.74 ± 0.05. The values are means ± S.E.M. of four to six quadruplicate experiments.

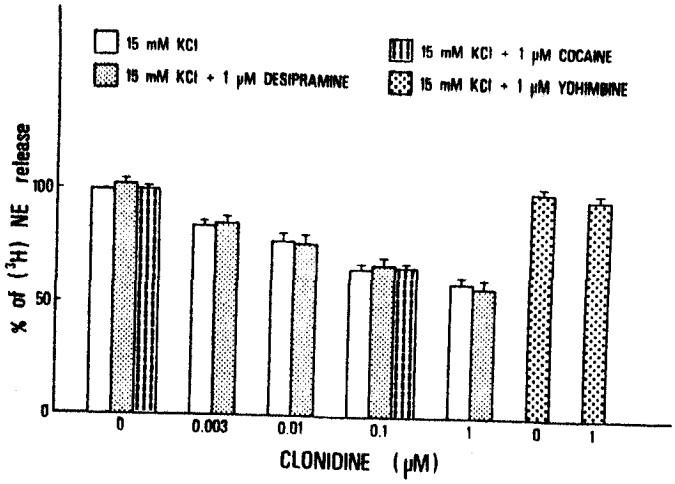

Fig. 3. Autoreceptor-mediated inhibition of [^{3}H]NE release of clonidine: effects of desipramine, cocaine and yohimbine. Rat cerebral cortex synaptosomes were labeled with [^{3}H]NE and depolarized in superfusion with 15 mM KCl as described under "Materials and Methods." The absolute value of fractional release evoked by high-K^+ (see "Materials and Methods") was 1.79 ± 0.13. The values are means ± S.E.M. of four to six experiments in quadruplicate.

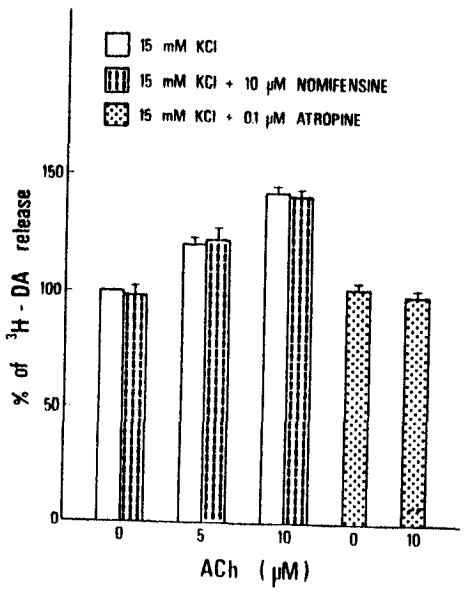

Fig. 4. Muscarinic-receptor mediated potentiation of [^{3}H]DA release by ACh: effects of nomifensine and of atropine. Rat striatal synaptosomes were labeled with [^{3}H]DA and depolarized in superfusion with 15 mM KCl as described under "Materials and Methods." The absolute value of fractional release evoked by high-K^+ (see "Materials and Methods") was 2.02 ± 0.11. The values displayed are means ± S.E.M. of three to five experiments in quadruplicate.

Potentiation by ACh of the [^{3}H]DA release elicited by 15 mM KCl in synaptosomes of the rat corpus striatum and study of the interaction between ACh and the DA uptake inhibitor nomifensine. Muscarinic heteroreceptors are localized on dopaminergic nerve endings in the corpus striatum. Their activation causes potentiation of the calcium-dependent evoked release of [^{3}H]DA (Lehmann and Langer, 1982; Raiteri *et al.*, 1982). The evoked release of [^{3}H]DA induced by 15 mM KCl in striatal synaptosomes labeled previously with the tritiated catecholamine was potentiated by exogenous ACh in a concentration-dependent manner (fig. 4). The action of 10 μM ACh was blocked by the muscarinic antagonist atropine (0.1 μM) which, by itself, did not modify the K^+-evoked [^{3}H]DA release. The action of ACh was unaffected by nomifensine, an inhibitor of DA uptake, added to the superfusion medium at 10 μM. At this concentration, the drug did not modify, by itself, the K^+-evoked release of [^{3}H]DA.

Inhibition by ACh of the [^{3}H]ACh release elicited by 15 mM KCl in synaptosomes of the rat cerebral cortex and study of the interaction between ACh and the choline uptake inhibitor hemicholinium-3. Muscarinic autoreceptors are present on cortical cholinergic nerve terminals to regulate the evoked ACh release (Szerb and Somogyi, 1973; Marchi *et al.*, 1983). The release of [^{3}H]ACh in superfused cortical synaptosomes was stimulated by a depolarizing concentration of KCl. In these experimental conditions, the evoked release of [^{3}H]ACh was shown to be almost totally calcium-dependent (Marchi *et al.*, 1983). In the experiments reported in figure 5 and table 1, we have investigated the possible interactions between muscarinic autoreceptors and the uptake of choline (ACh is not taken up by cholinergic terminals), in view of the well known relationships betwen high affinity choline uptake and ACh release (Kuhar and Murrin, 1978). Moreover, the absence of exchange phenomena between extra- and intraterminal ACh has made it possible to use ACh as a muscarinic autoreceptor agonist and therefore to study, in this case, the possible interactions between uptake blockade and presynaptic activity of the natural transmitter. Exogenous ACh decreased its own release in a concentration-dependent way (fig. 5). The action of 10 μM ACh was blocked by 0.1 μM atropine which, by itself, did not affect the K^+-evoked release of [^{3}H]ACh. The autoinhibition of [^{3}H]ACh release was not modified when the uptake carrier of choline was blocked with 100 μM hemicholinium-3. At the concentration used, hemicholinium-3, by itself, had no effect on the K^+-evoked release.

Neurotransmitter uptake in superfused synaptosomes and effects of neuronal uptake activation on presynaptic receptor function. The data presented so far show that, in superfused synaptosomes, the presence of neuronal uptake

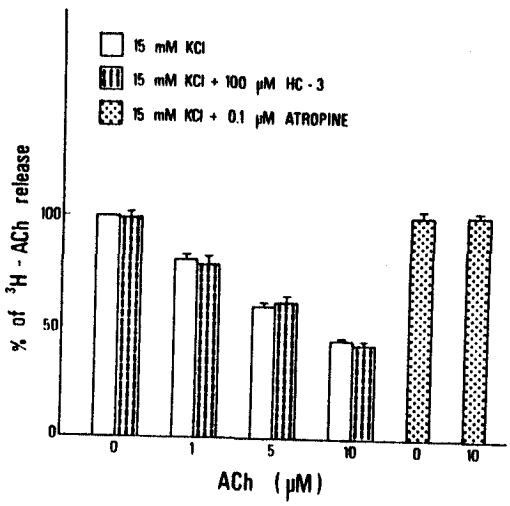

Fig. 5. Muscarinic-autoreceptors mediated inhibition of [^{3}H]ACh release by ACh: effects of hemicholinium-3 and of atropine. Rat cerebral cortex synaptosomes were labeled with [^{3}H]choline and depolarized in superfusion with 15 mM KCl as described under "Materials and Methods." The absolute value of fractional release evoked by high-K^+ (see "Materials and Methods") was 1.70 ± 0.14. The values are means ± S.E.M. of three to five experiments in quadruplicate.

inhibitors did not decrease the effectiveness of presynaptic receptor agonists. Inasmuch as in superfused synaptosomes reuptake of the released transmitters appears to be minimal (see Raiteri and Levi, 1978), the possibility existed that the hypothesized coupling between uptake mechanisms and presynaptic receptors could not be seen in our preparation. Experiments were therefore performed in order to ascertain; 1) if the integrity of the uptake mechanisms and their susceptibility to uptake inhibitors were maintained in our superfused synaptosomes preparation and 2) if, in order to show the existence of the above functional coupling, a potentially active uptake was not enough, but it was necessary to have the uptake system actually performing substrate transport.

The data reported in figure 6 show that cortical synaptosomes exposed in superfusion of 0.01 μM [^{3}H]-5-HT or to 0.1 μM [^{3}H] choline, in the presence of 15 mM KCl, accumulated tritium radioactivity. Under superfusion conditions, the uptake of [^{3}H] -5-HT and that of [^{3}H]choline were strongly inhibited by citalopram or by hemicholinum-3, respectively. Table 1 shows that the presence of uptake substrates (0.01 μM 5-HT, 0.1 μM tyramine or 0.1 μM choline) in the superfusion fluid did not cause any amelioration of the presynaptic receptor function. Furthermore, the effectiveness of presynaptic receptor agonists was not decreased by uptake inhibitors even when the uptake systems were activated.

Discussion

Experiments with brain slices have provided several examples showing that the regulation of transmitter release by exogenous presynaptic receptor agonists can be prevented by

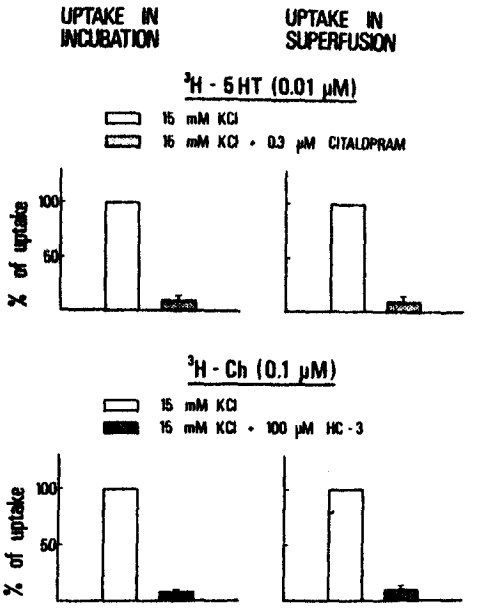

Fig. 6. Uptake of [^{3}H]-5-HT or of [^{3}H]choline in superfused or incubated synaptosomes and effects of the uptake inhibitors citalopram or hemicholinium-3. Rat cerebral cortex synaptosomes were either superfused or incubated 5 min at 37°C with 0.01 μM [^{3}H]-5-HT or 0.1 μM [^{3}H] choline, in a medium containing 15 mM KCl. The radioactive substrates were added concomitantly with KCl. Means ± S.E.M. of two experiments in triplicate are presented. The absolute values of uptake expressed as picomoles per milligram of protein per 5 min ± S.E.M. were 0.51 ± 0.05 for [^{3}H]-5-HT in incubation; 0.28 ± 0.03 for [^{3}H]-5-HT in superfusion; 3.30 ± 0.6 for [^{3}H]choline in incubation; and 1.44 ± 0.4 for [^{3}H]choline in superfusion.

inhibitors of the reuptake of the released transmitter. In particular: NE reuptake inhibitors counteracted the *alpha*-2 autoreceptor-mediated inhibition of the evoked [^{3}H]NE release by clonidine and related imidazolines (Starke and Montel, 1973; Pelayo *et al.*, 1980; Reichenbacher *et al.*, 1982; Göthert *et al.*, 1983); the 5-HT uptake inhibitor citalopram prevented the activation of 5-HT autoreceptors by LSD and the consequent inhibition of the evoked release of [^{3}H]-5-HT (Langer and Moret, 1982); the action of clonidine, as an inhibitor of the evoked [^{3}H]-5-HT release, was decreased by 5-HT reuptake inhibitors (Göthert *et al.*, 1983); the presynaptic actions of apomorphine on the evoked release of [^{3}H]DA or of [^{3}H]NE were counteracted by nomifensine, an inhibitor of DA reuptake (Cubeddu *et al.*, 1983) and by NE uptake blockers, respectively (Galzin *et al.*, 1982).

As already mentioned in the introduction, a number of explanations have been proposed for these findings. One explanation is limited to the interaction between imidazolines and NE uptake inhibitors: the latter, according to Pelayo *et al.* (1980), may compete for an imidazoline recognition site located on the *alpha*-2 autoreceptors. The following two explanations could be more generally applied to the various systems: 1) when reuptake inhibitors are present, the activity of presynaptic receptor agonists declines because the biophase concentration of released transmitter rises and, hence, the modulation elicited by the released transmitter becomes more pronounced (Reichenbacher *et al.*, 1982; Cubeddu *et al.*, 1983) and 2) reuptake inhibitors affect the activity of presynaptic receptor agonists because a functional link exists in nerve terminals between neuronal uptake and presynaptic receptors (Galzin *et al.*, 1982; Langer and Moret, 1982; Göthert *et al.*, 1983).

The last explanation appears rather provocative and bears a number of important implications. For instance, several transmitter reuptake inhibitors are antidepressants which, by themselves, interact weakly with transmitter receptors, but affect indirectly their "sensitivity" during long-term *in vivo* administration. Because a tonic autoreceptor-mediated inhibition of NE release seems to occur in the living brain (Langer, 1981; Starke, 1981; Cerrito and Raiteri, 1981), antidepressant uptake blockers would be expected to relieve it, thus increasing NE release. This together with the inhibition of NE uptake may contribute to the down regulation of the *beta* adrenoceptor-linked adenylate cyclase observed in the brain after long-term antidepressant treatment (Sulser *et al.*, 1978). Moreover, if the proposed functional link exists between uptake and presynaptic receptors, the activity of natural agonists could be strongly underestimated as the actions at presynaptic receptors of natural transmitter substances (*e.g.*, catecholamines or 5-HT) are currently studied in the presence of uptake inhibitors.

The possibility of multiple interpretations for the same findings originates from the complexity of the experimental system used, *i.e.*, brain slices in which the role of the transmitters released in the vicinity of the presynaptic receptors can only be inferred from indirect evidence. In a system of superfused synaptosomes, the transmitters released are rapidly removed: therefore they should not be recaptured by the uptake systems and should not accumulate in the biophase of presynaptic receptors, even in the presence of reuptake inhibitors. In these conditions, presynaptic receptor should be activated only by the exogenous agonists added to the superfusion fluid. In keeping with this view, neither uptake inhibitors nor presynaptic receptor antagonists, alone or in combination, increased trans-

mitter release in superfused synaptosomes (figs. 1–5; see also Raiteri and Levi, 1978), in contrast to what occurs in brain slices.

If the optimal functioning of presynaptic receptors depended on the existence of a functional link between these receptors and the uptake systems, one may expect presynaptic receptors to be less responsive in superfused synaptosomes in which reuptake is not operative. However, the results obtained with superfused synaptosomes (figs. 1–3), when compared with those obtained in brain slices (Langer and Moret, 1982; Göthert *et al.*, 1983), show that the effects of LSD and clonidine are quantitatively similar in the two preparations, suggesting that an operative uptake is not a prerequisite for the functioning of the presynaptic receptor systems.

This suggestion is indeed supported by the data shown in table 1. When the uptake of NE, of 5-HT or of choline in superfused synaptosomes was activated by adding the corresponding exogenous substrates to the superfusion medium, no improvement of the presynaptic receptor function could be observed. Furthermore, the addition of uptake inhibitors during activation of uptake with exogenous substrates did not decrease significantly the effectiveness of presynaptic receptor agonists. In table 1, particularly indicative appear to be the results obtained by activating uptake with tyramine (a substrate of the NE carrier without receptor agonist properties) and presynaptic autoreceptors with clonidine, in the absence or in the presence of the NE uptake inhibitor desipramine. This experimental situation was intended to mimick that present in a slice, but with the important difference that in our system the released NE could not increase in the presynaptic receptor biophase.

It has been reported that neuronal uptake inhibitors can interact with regulatory sites for the uptake which are distinct from the uptake site (see for instance Barbaccia *et al.*, 1983; Kennedy and Hanbauer, 1983). The possibility existed therefore that the functional linkage survives in superfused synaptosomes even in the absence of substrate for the carrier and that the presence of uptake inhibitors is necessary in order to abate it. However, this possibility does not seem to be supported by the results of figures 1 to 5. In fact, uptake inhibitors were ineffective in superfused synaptosomes even at high concentration ratios between uptake inhibitor and presynaptic receptor agonist, whereas in slices the various agonists were strongly counteracted already at equimolar concentrations.

Coming to the particular case of the interaction between imidazolines and NE uptake blockers, if these drugs interacted directly with clonidine at an imidazoline recognition site located on the *alpha*-2 autoreceptor (Pelayo *et al.*, 1980), one would expect desipramine or cocaine to inhibit the action of clonidine also in superfused synaptosomes. However, in this system, desipramine did not affect the inhibitory action of clonidine even when the ratio desipramine/clonidine was higher than 300, whereas in brain slices the imidazoline action was strongly depressed already at 1:1 ratio (Pelayo *et al.*, 1980; Göthert *et al.*, 1983).

In conclusion, our results show that the activity of presynaptic receptor agonists on the depolarization-evoked release of various transmitters was not affected by reuptake inhibitors when release was studied in conditions in which the accumulation of the transmitters released in the biophase of presynaptic receptors is minimized. The present data do not support therefore the hypothesis of the existence in central axon terminals of a functional link between uptake mechanisms and presynaptic receptors and the consequent possibility that neuronal uptake inhibitors affect, as an additional mechanism of action, the function of presynaptic receptors. Of course, a number of reasons besides that of the transmitter increasing in the biophase could be considered to explain the discrepancies between the results of the literature and our own results. For instance, the experiments with brain slices were all performed by using electrical stimulation, whereas superfused synaptosomes were depolarized with high-K^+. It should be noted, however, that the uptake of [^{3}H]-5-HT and [^{3}H]choline was largely maintained in the presence of 15 mM KCl. Another reason could be the removal of some endogenous modulators present in the living brain during the preparation of synaptosomes (and in brain slices) (see for example Barbaccia *et al.*, 1983) which could be responsible for the interactions between presynaptic receptor agonists and uptake inhibitors observed in slices.

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Send reprint requests to: Professor Maurizio Raiteri, Istituto di Farmacologia e Farmacognosia, Università di Genova, Viale Cembrano 4, 16148 Genova, Italy.