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False labelling of dopaminergic terminals in the rabbit caudate nucleus: uptake and release of [^{3}H]-5-hydroxytryptamine

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1 The effect of the catecholamine uptake inhibitor nomifensine and of the 5-hydroxytryptamine (5-HT) uptake blocker 6-nitroquipazine on the accumulation of [^{3}H]-5-HT (0.1 μM, 60 min incubation) and [^{3}H]-dopamine (0.1 μM, 30 min incubation) into slices of hippocampus and caudate nucleus of the rabbit was investigated. In addition, the influence of nomifensine on the electrically evoked [^{3}H]-5-HT release from caudate nucleus slices and of nomifensine and 6-nitroquipazine on [^{3}H]-5-HT released from caudate nucleus slices was analysed.

2 In hippocampal slices, which contain practically no dopaminergic but densely distributed 5-hydroxytryptaminergic and noradrenergic nerve terminals (ratio of dopamine:5-HT:noradrenaline about 1:30:25), nomifensine (1, 10 μM) did not affect the accumulation of [^{3}H]-5-HT; 6-nitroquipazine (1 μM) reduced [^{3}H]-5-HT uptake to about 35% of controls. In the caudate nucleus, however, where dopamine is the predominant monoamine (ratio of dopamine:5-HT:noradrenaline about 400:25:15) nomifensine (1, 10 μM) reduced the tritium accumulation to 65% whereas 6-nitroquipazine (1 μM) was ineffective. The combination of both drugs (1 μM each) led to a further decrease to about 15%.

3 The uptake of [^{3}H]-dopamine into hippocampal slices was blocked by both nomifensine (1 μM) and 6-nitroquipazine (1 μM) whereas in caudate nucleus slices only nomifensine (1, 10 μM) reduced the accumulation of [^{3}H]-dopamine. The combination of both drugs was not more effective than nomifensine alone. The different effects of both uptake inhibitors in the hippocampus and caudate nucleus suggest a neurone specific rather than a substrate specific mode of action.

4 In caudate nucleus slices incubated with [^{3}H]-5-HT and superfused continuously the electrically evoked 5-HT release was diminished by the D_2-dopamine receptor agonist LY 171555 and enhanced by the D_2-receptor antagonist domperidone. If, however, the labelling of caudate nucleus slices was performed in the presence of 1 μM or 10 μM nomifensine, the modulation of 5-HT release via D_2-receptors was reduced or abolished, respectively. In the hippocampus both LY 171555 and domperidone were completely ineffective in modulating 5-HT release regardless of the absence or presence of nomifensine.

5 The present results indicate that an inverse cross labelling of [^{3}H]-5-HT into dopaminergic and of [^{3}H]-dopamine into 5-hydroxytryptaminergic terminals may occur despite the low concentration (0.1 μM) of tritiated transmitters used. Such cross labelling, as demonstrated with the incubation period of 60 min in the caudate nucleus, may falsely indicate the existence of D_2-dopamine receptors modulating [^{3}H]-5-HT release. If both 5-hydroxytryptaminergic and dopaminergic terminals are present within the brain region under investigation false labelling can be corrected using neuronally specific uptake inhibitors.

Introduction

In contrast to hippocampal noradrenaline release, hippocampal 5-hydroxytryptamine (5-HT) release, is not modulated by D_2-dopamine receptors (Jackisch *et al.*, 1985). This apparent lack of dopamine receptors on 5-hydroxytryptaminergic nerve terminals in the hippocampus may not necessarily also be true for other brain regions, e.g. the caudate nucleus, where presynaptic 5-hydroxytryptamine-dopamine in-

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terrelations could play an important role. It is well known that the 5-hydroxytryptamine neurones of the dorsal raphé nucleus have their terminal field in the striatum whereas the mesolimbic 5-hydroxytryptaminergic system predominantly corresponds to the neurones of the median raphé nucleus. The 5-hydroxytryptaminergic dorsal and median raphé nuclei, however, constitute two anatomically and functionally distinct cell groups (Lee & Geyer, 1983), possibly with a different pattern of presynaptic receptors in their respective terminal fields.

Therefore, we were interested in the possibility of a dopaminergic modulation of 5-HT release within the caudate nucleus. In this tissue a further difference between it and the hippocampus may play a decisive role: dopamine is the predominant transmitter in the caudate nucleus and is present at a much higher concentration than striatal 5-HT (Starke *et al.*, 1978; Limberger & Hedler, unpublished results), whereas the dopamine concentration in the hippocampus is nearly negligible in comparison to the levels of 5-HT and noradrenaline (Strittmatter *et al.*, 1982; Juorio & Greenshaw, 1985).

Consequently, we first investigated the uptake of both monoamines, [^{3}H]-5-HT and [^{3}H]-dopamine, into rabbit caudate and hippocampal slices before studying the release of [^{3}H]-5-HT in the caudate nucleus in comparison to the hippocampus. Generally a concentration of 0.1 μM of the tritiated transmitter is used for incubation of brain slices in order to label selectively the corresponding nerve terminals (Shaskan & Snyder, 1970; Starke *et al.*, 1978; Schlicker *et al.*, 1984). Despite the low, supposedly 'specific' concentration of 0.1 μM of the labelled transmitter used, we were aware of a possible non-specific uptake of monoaminergic transmitters. This has been shown histochemically for dopamine by Berger & Glowinsky (1978) in the rat cortex, and by Kelly *et al.* (1985) in the rat substantia nigra. In addition, Kelly and coworkers (1985) also suggested a false labelling by [^{3}H]-5-HT of striatal synaptosomes of the rat, but this finding was not followed up by functionally relevant release studies. Furthermore, the incubation time for the uptake of tritiated monoamines into brain slices preceding release experiments usually is of longer duration than that for the labelling process of synaptosomes (Kelly *et al.*, 1985). Therefore, the incubation time used for the release studies was also used in the uptake experiments. The tissue radioactivity remaining after the relatively long incubation period and wash represents the net result of uptake, degradation and release of the amine or the metabolites; therefore it does not allow the measurement of the initial rate of uptake.

Some of the present results were presented at the Joint Meeting of the Belgian, Dutch, and German Pharmacological and Toxicological Societies, Aachen, September 1985 (Feuerstein & Hertting, 1985).

Methods

Rabbits of either sex weighing 1.7–2.7 kg were decapitated. The brain was quickly removed and the hippocampi or the caudate nuclei bluntly detached from their surroundings. Hippocampal slices (0.4 mm thick, 5–7 mm diameter, 5–7 mg wet weight) and slices of the head of the caudate nucleus (0.3 mm thick, 3–4 mm diameter, 2–3 mg wet weight) were prepared using a McIllwain tissue chopper.

Uptake experiments

The incubation procedure consisted of 3 periods: (1) 15 min of pre-incubation in medium (37°C) with or without drugs, (2) incubation in the presence of tritiated transmitters (0.1 μM each, 30 min for [^{3}H]-dopamine, 27 Ci mmol^{-1}; 60 min for [^{3}H]-5-HT, 22–27 Ci mmol^{-1}), and (3) a 30 min period of discontinuous washing in the presence of the uptake inhibitor (if indicated): after 10 and 20 min the slices were washed once with 15 ml fresh medium followed by re-addition of 10 ml fresh medium. At the end of the washing period each slice was dissolved in 0.5 ml Soluene-350 (Packard Instruments, Frankfurt, FRG) for tritium determination. The tritium content of slices incubated and treated as above, but at 0°C was about 6% of the tritium content of slices incubated at 37°C. This small amount of tritium accumulation at 0°C, probably due to unspecific adsorption, was not subtracted from the values at 37°C.

Superfusion experiments

Superfusion and electrical stimulation of rabbit hippocampal or caudate nucleus slices preincubated with [^{3}H]-5-HT were performed essentially as described recently (Feuerstein *et al.*, 1985a) with only slight modifications. In brief: the slices were incubated as above but without the period of discontinuous washing. They were then rinsed, transferred to glass superfusion chambers, superfused with medium prewarmed to 37°C and saturated with 5% CO_2/95% O_2, pH 7.4, at a rate of 1 ml min^{-1}. After 45 min of continuous washing the superfusate was collected in 5 min samples for tritium determination by liquid scintillation counting. The composition of the incubation and superfusion medium was (mmol l^{-1}): NaCl 118, KCl 4.8, $CaCl_2$ 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, ascorbic acid 0.57, disodium EDTA 0.03.

During superfusion the slices were electrically stimulated twice (for 2 min each; rectangular unipolar pulses: 2 ms, 3 Hz; 5 V cm^{-1}, 24 mA) after 60 min (S_1)

and 125 min (S_2). Drugs to be tested were added to the medium from 15 min before S_2 onwards. None of them affected basal tritium outflow. In some experiments nomifensine 1 μM was present in the medium throughout the superfusion. Controls without drugs given before S_2 were always run in parallel to drug experiments. At the end, the slices were removed from the chambers and dissolved in Soluene 350 for tritium determination.

Separation of [^{3}H]-5-HT from its metabolite [^{3}H]-5-hydroxyindole acetic acid (5-HIAA) and of [^{3}H]-dopamine from its deaminated metabolites in superfusate samples from caudate slices was performed using the separation method described by Steppeler *et al.* (1982). The slices were electrically stimulated twice: the first stimulation was carried out in the absence of 6-nitroquipazine, whereas the 5-HT uptake blocker was added to the superfusion fluid from 55 min before the second stimulation until the end of superfusion. The 5 min samples of the superfusate before and during each stimulation and two respective samples after each stimulation were analysed for the unmetabolized transmitters and their deaminated metabolites. The recovery from standard samples of [^{3}H-5-HT and [^{3}H]-dopamine was 93.9 ± 3.0%, $n = 8$, and 90.7 ± 2.2%, $n = 4$, respectively. The data were not corrected for recovery, which, according to the law of error propagation, would have increased the variances of the data without improving the experimental information.

Evaluation and statistics

The fractional rate of tritium outflow $(5\,\text{min})^{-1}$ and the stimulation evoked overflow of tritium were expressed as % of the tritium content of the tissue at the onset of the respective stimulation period. Drug effects were evaluated by calculating the ratio (S_2/S_1) of the evoked overflow of the two stimulation periods (Feuerstein *et al.*, 1985a).

All results (tritium contents on uptake experiments and S_2/S_1 ratios) were expressed as means ± s.e.mean. The existence of differences between the means of treatments and their corresponding controls was tested with a one-way analysis of variance; the preconditions of this analysis of variance were proved using Bartlett's test with a minimum level of significance of 10% to refute the null hypothesis (homogeneity of variances, goodness-of-fit for normal distribution). Student's t test (two-tailed) was used subsequently to determine the significance of differences between the groups of treatments, employing the error mean square of the analysis of variance to an estimate of the standard deviation of all means. When results are expressed as % of corresponding controls, the law of error propagation was respected for calculation of the s.e.means.

Drugs used

[1,2-^{3}H(N)]-5-hydroxytryptamine creatinine sulphate and [7-^{3}H]-3,4-dihydroxyphenylethylamine were obtained from NEN, Dreieich, FRG. The following drugs were kindly provided or acquired as follows: 5-hydroxytryptamine creatinine sulphate (Sigma, München, FRG); nomifensine hydrogen maleate (Hoechst, Frankfurt, FRG); 6-nitroquipazine maleate (Duphar, Weesp, The Netherlands); (−)4,4a,5,6,-7,8,8a,9-octahydro-5-n-propyl-2H-pyrazolo-3,4-g-quinoline HCl (LY 171555; Eli Lilly, Indianapolis, IN, USA); domperidone (Janssen, Beerse, Belgium). All other chemicals were of analytical grade from commercial sources.

Stock solutions of drugs were freshly prepared in water or medium for each experiment with the exception of domperidone which was dissolved in 10 mM citric acid.

Results

[^{3}H]-5-hydroxytryptamine uptake experiments

It is evident from Figure 1 that the specific 5-HT uptake blocker 6-nitroquipazine (1 μM; Classen *et al.*,

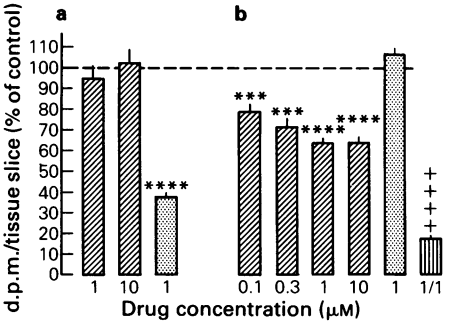

Figure 1 Effects of nomifensine (oblique hatched columns), 6-nitroquipazine (stippled columns) and 6-nitroquipazine in the presence of nomifensine (vertical hatched columns) on the tritium content (^{3}H-total measured as d.p.m.) of hippocampal (a) and caudate nucleus (b) slices of the rabbit. The slices were preincubated for 15 min in the absence and presence of the uptake blockers under investigation, incubated with [^{3}H]-5-HT for 60 min and discontinously washed for a further 30 min. The concentrations used are indicated under the columns; when both 6-nitroquipazine and nomifensine were combined a concentration of 1 μM for each (1/1) was used. Each column represents the mean and vertical bars s.e.mean. ($n = 9$–16 for each group). ***$P < 0.001$; ****$P < 0.0001$; significantly different from corresponding controls (100%). $^{++++}P < 0.0001$, compared to the tritium content in the presence of nomifensine 1 μM (oblique hatched column).

1984) effectively diminished the tritium uptake into hippocampal slices incubated with 0.1 μM [^{3}H]-5-HT, whereas the catecholamine uptake inhibitor nomifensine (1, 10 μM) was ineffective in this respect. The tritium accumulation in caudate nucleus slices incubated with [^{3}H]-5-HT was reduced in a concentration-dependent manner by nomifensine (significant differences from respective controls: see legend to Figure 1, and between the nomifensine groups: $P<0.01$), but not by 6-nitroquipazine. Concentrations of 6-nitroquipazine higher than 3.2 μM decreased the tritium accumulation within caudate nucleus slices incubated with [^{3}H]-5-HT (not shown). In the presence of 1 μM nomifensine, however, 6-nitroquipazine (1 μM) strongly diminished the tritium accumulation.

[^{3}H]-dopamine uptake experiments

Figure 2 shows that nomifensine diminished the tritium accumulation in both hippocampal and caudate nucleus slices incubated with [^{3}H]-dopamine. Also 6-nitroquipazine reduced the uptake of tritium into hippocampal slices, but did not change the tritium content of caudate nucleus slices. 6-Nitroquipazine (1 μM), applied in addition to nomifensine (1 μM) (which *per se* decreased the uptake of [^{3}H]-dopamine) significantly reduced the already diminished tritium uptake into hippocampal slices but did not further diminish the tritium content of caudate nucleus slices. With caudate nucleus slices 6-nitroquipazine (1 μM) again was not effective when the uptake of [^{3}H]-dopamine was maximally reduced by nomifensine up to 32 μM (not shown).

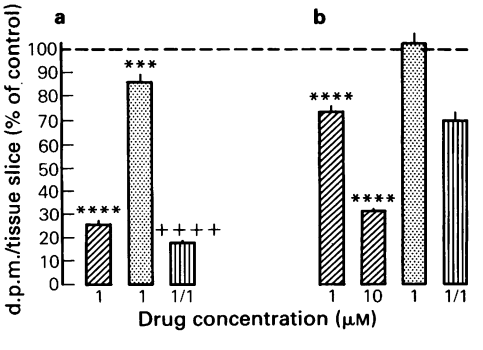

Figure 2 Effects of nomifensine (oblique hatched columns), 6-nitroquipazine (stippled columns) and 6-nitroquipazine in the presence of nomifensine (vertical hatched columns) on the tritium content (^{3}H-total measured as d.p.m.) of hippocampal (a) and caudate nucleus (b) slices of the rabbit incubated with [^{3}H]-dopamine for 30 min. Further conditions were the same as those described in the legend of Figure 1. For explanation of symbols used see legend of Figure 1.

Evoked release of tritium from brain slices incubated with [^{3}H]-5-hydroxytryptamine

In hippocampal slices, incubated with [^{3}H]-5-HT, then superfused and electrically stimulated twice, neither the dopamine D_2-receptor agonist LY 171555 (1 μM; Jackisch *et al.*, 1985) nor the D_2-receptor antagonist domperidone (0.1 μM) modulated the tritium overflow in the absence or presence of nomifensine (1 μM) during the superfusion or during the incubation and superfusion (Figure 3).

However, LY 171555 markedly diminished and domperidone strongly enhanced the electrically evoked ^{3}H-overflow from caudate nucleus slices incubated with [^{3}H]-5-HT in the absence of nomifensine and superfused in the presence of nomifensine (1 μM). If nomifensine (1 or 10 μM) was present during the tissue incubation with [^{3}H]-5-HT, it diminished the modulation of the electrically evoked tritium overflow by both LY 171555 and domperidone in a concentration-dependent manner (Figure 4).

It should be noted that the S_2/S_1-ratio of the control group (no drug given before S_2 onwards) of caudate nucleus slices labelled with [^{3}H]-5-HT in the absence of nomifensine was 0.720 ± 0.030, $n = 6$ and significant-

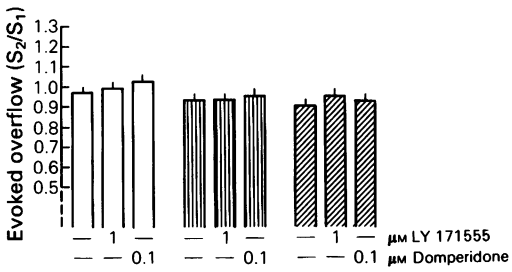

Figure 3 Effects of LY 171555 and domperidone on the evoked overflow of tritium from hippocampal slices incubated with [^{3}H]-5-hydroxytryptamine, superfused and electrically stimulated twice (S_1, S_2). Nomifensine (1 μM) was either absent (open columns) or present during superfusion (vertical hatched columns) or during preincubation, incubation and superfusion (oblique hatched columns), respectively. LY 171555 or domperidone was added to the superfusion medium 15 min before S_2 at the concentrations indicated. Ordinate scale: ratio between the overflow of tritium evoked by S_2 and the overflow evoked by S_1 (S_2/S_1). The tritium overflow of S_1 (expressed as % of tissue tritium) amounted to 2.75 ± 0.10% ($n = 12$) in the absence of nomifensine, to 2.97 ± 0.09% ($n = 14$) in the presence of nomifensine during superfusion and to 2.49 ± 0.10% ($n = 12$) in the presence of nomifensine during preincubation, incubation and superfusion. Each column represents the mean and vertical bars s.e.mean ($n = 4-5$ for each group). There were no significant differences between any of the groups.

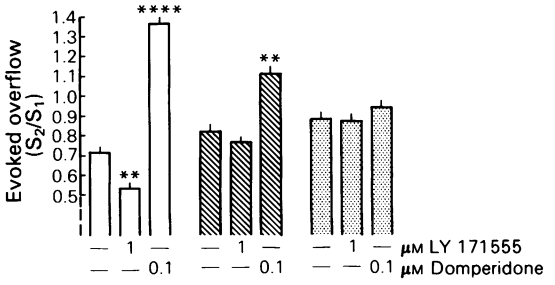

Figure 4 Effects of LY 17155 and domperidone on the evoked overflow of tritium from caudate nucleus slices incubated with [^{3}H]-5-hydroxytryptamine, superfused with medium containing nomifensine 1 μM and electrically stimulated twice (S_1, S_2). Nomifensine was either absent (open columns) or present during preincubation and incubation at a concentration of 1 μM (hatched columns) or 10 μM (stippled columns), respectively. Other conditions were the same as those described in the legend of Figure 3. The tritium overflow of S_1 (expressed as % of tissue tritium) amounted to 5.60 ± 0.23% ($n = 18$) in the absence of nomifensine, to 2.99 ± 0.07% ($n = 20$) in the presence of nomifensine 1 μM during preincubation and incubation and to 2.95 ± 0.12% ($n = 12$) in the presence of nomifensine 10 μM during preincubation and incubation, $^{**}P < 0.01$; $^{***}P < 0.0001$; significantly different from corresponding controls.

ly lower than the respective S_2/S_1-ratio of caudate nucleus slices incubated in the presence of 10 μM nomifensine: 0.883 ± 0.037, $n = 4$, $P < 0.01$.

Effect of 6-nitroquipazine on the metabolism of [^{3}H]-5-hydroxytryptamine and [^{3}H]-dopamine within the caudate nucleus

As shown in Figure 5a, 6-nitroquipazine (1 μM) did not change the fraction of [^{3}H]-5-HT in the basal tritium outflow but markedly enhanced the percentage of the unmetabolized transmitter during and after the electrical stimulation. The total tritium outflow and stimulation evoked ^{3}H-overflow before, during and after stimulation were decreased in the presence of 6-nitroquipazine (tritium overflow during the first stimulation period: 27364 ± 1477 d.p.m., $n = 5$, during the second stimulation period: 19258 ± 689 d.p.m., $n = 5$, $P < 0.01$). This is in contrast to the much lower S_2- versus S_1-values of the control group of release experiments occurring during incubation of the caudate nucleus in the absence of nomifensine (see legend of Figure 4). Nomifensine, applied during the incubation (10 μM) and superfusion (1 μM), strongly reduced both basal and evoked tritium overflow (Figure 5b). In addition, it modified the effects of 6-nitroquipazine on the evoked tritium overflow: in comparison to the effect of 6-

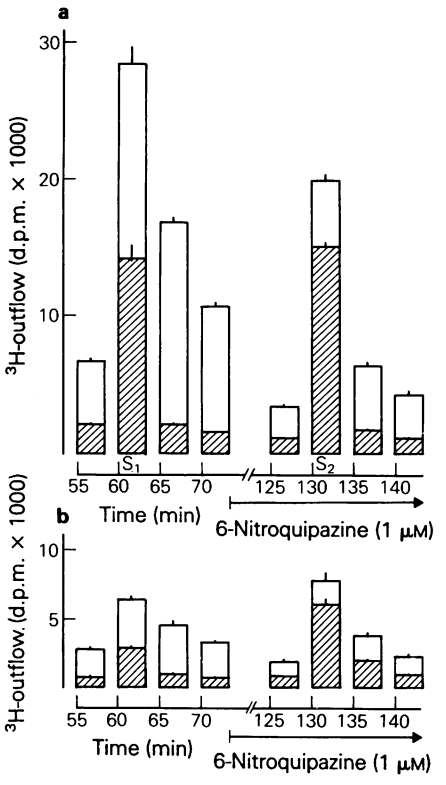

Figure 5 Effect of 6-nitroquipazine on the outflow of tritium and [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) from slices of rabbit caudate nucleus incubated with [^{3}H]-5-HT, superfused and electrically stimulated twice (S_1, S_2). Nomifensine was absent throughout the experiment (a) or present during preincubation and incubation at a concentration of 10 μM as well as during superfusion at 1 μM (b). 6-Nitroquipazine was added 55 min before S_2. Abscissa scale: time elapsed since the beginning of superfusion (min). Ordinate scale: tritium outflow (d.p.m. per 5 min); [^{3}H]-5-HT as % of total tritium is indicated in the columns by the solid area. Each column represents the mean and vertical bars s.e.mean, $n = 4$.

nitroquipazine alone (Figure 5a), the total ^{3}H-overflow of the second stimulation period was no longer diminished but enhanced compared with that of the first stimulation period (5815 ± 219 d.p.m., $n = 5$ (first stimulation) vs. 7185 ± 443 d.p.m., $n = 5$ (second stimulation), $P < 0.05$). Although nomifensine reduced the total amount of ^{3}H-overflow, it did not change the percentage of [^{3}H]-5-HT in the basal tritium outflow and the stimulation evoked overflow, but increased the percentage of [^{3}H]-5-HT in the two post-stimulation collection periods. In the presence of 6-nitroquipazine in addition to nomifensine, the unmetabolized [^{3}H]-5-HT was found to be markedly

Table 1 Effect of 6-nitroquipazine on the percentage of [^{3}H]-dopamine in the total tritium efflux from caudate nucleus slices incubated with [^{3}H]-dopamine

	Tritium efflux (5 min)$^{-1}$ in the absence of 6-nitroquipazine				*Tritium efflux (5 min)$^{-1}$ in the presence of 6-nitroquipazine*			
	Before	*During*	*After S_1*		*Before*	*During*	*After S_2*	
Time of superfusion (min)	55–60	60–65	65–70	70–75	125–130	130–135	135–140	140–145
Total ^{3}H (d.p.m.)	2838 ±120	11198 ±570	9564 ±275	6359 ±226	2265 ±60	7560 ±511	7466 ±146	5330 ±130
[^{3}H]-dopamine (%)	9.0 ±0.7%	24.5 ±1.6%	3.7 ±0.2%	3.7 ±0.2%	7.5 ±0.4%	21.3 ±1.5%	4.8 ±0.4%	3.7 ±0.1%

The slices were superfused with either drug-free medium for 75 min or with medium containing 6-nitroquipazine (1 μM) for the rest of the experiment. They were electrically stimulated twice at 60 min (S_1) and 130 min (S_2) during the superfusion. Data shown are means ± s.e.mean of 4 experiments.

increased in all collection periods compared to that in the presence of nomifensine alone.

In caudate nucleus slices incubated with [^{3}H]-dopamine, 6-nitroquipazine (1 μM) did not change the fraction of deaminated metabolites in either the basal tritium outflow or the evoked tritium overflow before, during and after stimulation (Table 1).

Discussion

The presence of inhibitory 5-HT receptors on dopamine terminals in the striatum of the rat has been described by Ennis *et al.* (1981). In contrast, there seems to be no report about the modulation of 5-HT release by dopamine receptors. In hippocampal tissue, where the density of dopamine terminals is very low (Strittmatter *et al.*, 1982) neither dopamine, LY 171555, a D_2-receptor agonist (Jackisch *et al.*, 1985; Figure 3), nor the D_2-receptor antagonist domperidone (Figure 3) influenced the evoked 5-HT release. However, the present experiments show that under similar conditions 5-HT release from caudate slices was decreased by LY 171555 and increased by domperidone, indicating a possible modulation of 5-HT release by presynaptic D_2-receptors.

In contrast to the hippocampus, the tissue content of dopamine in the caudate nucleus greatly exceeds the concentration of 5-HT (Starke *et al.*, 1978; Limberger & Hedler, unpublished results) which most probably reflects the density of the respective nerve terminals. Therefore, if 5-HT is taken up and subsequently released from dopamine terminals a modulation by dopamine autoreceptors (Starke *et al.*, 1983) could explain the phenomena observed.

'False' labelling of 5-HT terminals by dopamine was described by Berger & Glowinski (1978). The observation that 5-HT uptake into synaptosomes from rat striatum was partially inhibited (Hunt *et al.*, 1974; Schacht & Heptner, 1974) by the catecholamine uptake inhibitor nomifensine suggests that 5-HT may also be taken up into structures other than 5-hydroxytryptaminergic nerve endings (Kelly *et al.*, 1985). In our experiments nomifensine failed to affect [^{3}H]-5-HT uptake into hippocampal slices but significantly decreased [^{3}H]-5-HT accumulation of caudate nucleus slices. On the other hand, the 5-HT uptake blocker 6-nitroquipazine (Classen *et al.*, 1984) inhibited [^{3}H]-5-HT uptake into hippocampal but not into caudate nucleus tissue. Only the combination of both uptake inhibitors diminished very markedly the accumulation of [^{3}H]-5-HT into caudate nucleus slices.

We interpret the observed phenomena as follows: both nomifensine and 6-nitroquipazine inhibited the transport of monoamines selectively into the appropriate neurones, i.e. nomifensine blocked the uptake of both monoamines into dopaminergic and 6-nitroquipazine into 5-hydroxytryptaminergic terminals, but not *vice versa*. Thus, these drugs seem to act in a neurone specific rather than substrate specific manner. This can be clearly seen in hippocampal tissue where nomifensine was ineffective in diminishing [^{3}H]-5-HT uptake.

In caudate nucleus slices [^{3}H]-5-HT was taken up not only into the relatively small 5-hydroxytryptaminergic compartment but also into the much larger compartment of dopaminergic nerve endings. Here, nomifensine inhibited the uptake of [^{3}H]-5-HT maximally to about 35% of controls and 6-nitroquipazine, when given alone, seemed to be ineffective. However, the combination of both drugs inhibited the [^{3}H]-5-HT accumulation by about 85%.

The apparent ineffectiveness of 6-nitroquipazine in caudate nucleus slices when given alone may have been due to the following reason: although this drug blocked the specific [^{3}H]-5-HT uptake into 5-hydroxy-

tryptaminergic nerve endings its effect was masked by a shift of [^{3}H]-5-HT accumulation into dopamine terminals and became visible only under the combined treatment if this shift was prevented by nomifensine. It has to be noted that the degree of [^{3}H]-5-HT uptake inhibition by 6-nitroquipazine in both tissues was about the same: compare the relative decrease from 100% to about 35% in the hippocampus to the decrease from 65% (nomifensine 1 μmol l^{-1}) to 15% (nomifensine 1 μmol l^{-1} plus 6-nitroquipazine 1 μmol l^{-1}) in caudate nucleus tissue.

This suggested false labelling of dopaminergic terminals was confirmed functionally. When the false uptake of [^{3}H]-5-HT into dopamine terminals was prevented by nomifensine during the incubation period of caudate nucleus slices, the apparent modulation of 5-HT release via D_2-receptors disappeared. In the hippocampus, where no such false labelling occurred, a D_2-receptor mediated modulation of 5-HT release was not detectable even in the absence of nomifensine. Thus, in both brain regions D_2-receptors do not modulate 5-HT release from 5-hydroxytryptaminergic terminals.

In the hippocampus the S_2/S_1 ratio of controls (no drug given before S_2) was near 1 despite the absence or presence of nomifensine during the superfusion or during the incubation and superfusion. However, using caudate nucleus slices the S_2/S_1 ratio of controls was about 0.7 (Figure 4) when [^{3}H]-5-HT had been allowed to accumulate in the absence of nomifensine and close to 0.9 if the false labelling had been prevented by nomifensine. This was in contrast to a marked decrease of the S_1 values from nomifensine-treated tissue (see legend of Figure 4). The observed phenomenon can be explained by a more rapid exhaustion of the falsely located transmitter.

Blockade of the re-uptake by a specific uptake inhibitor applied before S_2 normally increased the S_2/S_1 ratio as shown for 6-nitroquipazine on 5-HT release in the hippocampus (Feuerstein *et al.*, 1985a). This was also the case for the evoked tritium outflow and the respective [^{3}H]-5-HT fraction (Figure 5b) if false labelling was prevented. In contrast, 6-nitroquipazine failed to display this effect in caudate slices where [^{3}H]-5-HT presumably had also entered dopamine terminals.

A possible inhibition of monoamine oxidase activity by 6-nitroquipazine described by Classen *et al.* (1984) has to be considered to participate in the mechanism of action of this drug. Such a property, however, could not be demonstrated with dopamine as substrate (Table 1). The increase of the non-deaminated [^{3}H]-5-HT fraction of the stimulus evoked ^{3}H-overflow from caudate nucleus slices following 6-nitroquipazine is expected to be caused mainly by prevention of the re-uptake of the released transmitter (Classen *et al.*, 1984; Feuerstein *et al.*, 1985b). The additional increase of the non-deaminated [^{3}H]-5-HT fraction in the two post-stimulation periods due to nomifensine may be explained accordingly by the prevention of the uptake of [^{3}H]-5-HT into, and metabolism within the dopaminergic terminals.

[^{3}H]-dopamine uptake into hippocampal slices was inhibited by both nomifensine and 6-nitroquipazine. This is in good agreement with the observation of Berger & Glowinsky (1978) of a false labelling of noradrenergic and 5-hydroxytryptaminergic nerve endings by [^{3}H]-dopamine: nomifensine is expected to inhibit the transport into noradrenergic terminals whereas 6-nitroquipazine inhibits the uptake of [^{3}H]-dopamine into 5-HT terminals. The combined treatment yielded a further decrease of the tissue content of [^{3}H]-dopamine.

In caudate tissue, however, [^{3}H]-dopamine uptake was inhibited by nomifensine but not by 6-nitroquipazine either if given alone or in combination with nomifensine. This ineffectiveness of 6-nitroquipazine in diminishing the uptake of [^{3}H]-dopamine into caudate slices is not surprising in view of the predominant dopaminergic versus the sparse 5-hydroxytryptaminergic innervation of this tissue, which probably hindered the significant demonstration of the false labelling of 5-HT terminals in this structure.

In summary, the results of this study demonstrate the need for prevention of false labelling of dopaminergic terminals when a [^{3}H]-5-HT release model is used. Conversely, despite the different density of dopaminergic and 5-hydroxytryptaminergic terminals in the caudate nucleus the possibility of the false uptake of [^{3}H]-dopamine into 5-HT terminals should be considered in release studies using caudate nucleus slices labelled with tritiated dopamine.

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