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Induction of 5-hydroxytryptamine release by tramadol, fenfluramine and reserpine

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Abstract

Tramadol is a centrally acting analgesic with several modes of action. Enhancement of 5-hydroxytryptamine release contributes to its actions. We investigated in which way tramadol induces 5-hydroxytryptamine release. Rat brain frontal cortex slices were preincubated with [^{3}H]5-hydroxytryptamine, then superfused using conditions which impaired either carrier mediated release or exocytosis. Tramadol (10 and 100 μM), fenfluramine (1 μM) and reserpine (10 μM) enhanced the basal release of [^{3}H]5-hydroxytryptamine. In the presence of a high concentration of 6-nitroquipazine effects of tramadol were reduced and those of fenfluramine abolished. Effects by reserpine were enhanced, indicating that [^{3}H]5-hydroxytryptamine depletion was counteracted by reuptake. When NaCl was replaced by LiCl, tramadol did not affect [^{3}H]5-hydroxytryptamine release, fenfluramine induced a small and reserpine a marked facilitation. Omission of $CaCl_2$ did not alter fenfluramine and reserpine effects while those by tramadol were reduced. It is concluded that tramadol induces both carrier mediated 5-hydroxytryptamine release as well as exocytosis.

Keywords: 5-HT (5-Hydroxytryptamine, serotonin) release; Carrier mediated release; Exocytosis; Fenfluramine; Reserpine; Tramadol

1. Introduction

Tramadol is a centrally acting analgesic with several modes of action, activation of opioid receptors (Hennies et al., 1988) preferentially of the μ-subtype (Raffa et al., 1992), and enhancement of the extraneuronal concentration of the monoamine neurotransmitters noradrenaline and 5-hydroxytryptamine (5-HT) by interference with the uptake and release mechanisms (Driessen and Reimann, 1992; Raffa et al., 1992; Driessen et al., 1993). As regards 5-HT, tramadol has been shown to reduce the synaptosomal uptake of [^{3}H]5-HT (Raffa et al., 1992); however, unlike the 5-HT uptake inhibitor 6-nitroquipazine which enhances mainly the stimulation-evoked overflow of tritium from brain slices preincubated with [^{3}H]5-HT, tramadol enhances mainly the basal outflow of tritium, thus, mimicking uptake inhibition under conditions, when only accumulation is determined. However, the latter effect is almost abolished when slices are superfused in the presence of a high concentration of 6-nitroquipazine (Driessen and Reimann, 1992). This finding provides evidence for an action like an indirect mimetic or like an activation of a carrier mediated outward transport of the neurotransmitter (Levi and Raiteri, 1993).

The present study characterises the interaction of tramadol with the serotonergic terminal further. We compared the effects of tramadol to reference substances which also induce release of 5-HT, under conditions, when its deamination is blocked, namely fenfluramine, known to induce release by inverse activation of the plasma membrane bound, Na^+-dependent 5-HT carrier (Maura et al., 1982), and reserpine, which enhances cytoplasmic 5-HT concentrations by blocking the H^+-dependent vesicular transporter, and thereby enhancing 5-HT outflow. Experiments were carried out either under normal conditions, or conditions interfering with exocytosis or carrier mediated release.

2. Materials and methods

2.1. Experimental procedure

Male Sprague–Dawley rats weighing 230–380 g were killed by decapitation. The brain was quickly removed and chilled. After removal of the superficial layer (0.3 mm),

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0.4 mm thick slices (5 mm diameter) of the frontal cortex were cut as described and characterised previously (Reimann et al., 1981). The slices were preincubated with 0.1 μM [^{3}H]5-HT at 37°C for 30 min, then transferred to glass superfusion chambers and superfused with medium at a rate of 1 ml min^{-1} for 105 min at 37°C. Fractions of the superfusate were collected for consecutive periods of 5 min, starting 50 min after onset of superfusion. The medium used for incubation and superfusion was composed of (mM): 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; it was saturated with 5% CO_2 in O_2, and the pH was adjusted to 7.4 by adding NaOH. Pargyline, 10 μM, was added to the medium throughout superfusion. In part of the experiments, NaCl was replaced by an equimolar amount of LiCl, or $CaCl_2$ was omitted during superfusion. Drugs were either present throughout superfusion, as indicated, or added after 75 min of superfusion.

At the end of the experiment, the slices were solubilised in 0.5 ml Soluene 350 (Packard). The radioactivity in superfusates and slices was measured by liquid scintillation spectrometry after addition of the appropriate scintillation fluids. The counting efficiency was determined with either internal standards or external standardization.

2.2. Calculations, statistics

The fractional rate of tritium outflow was calculated by dividing the tritium content in the superfusate by the tritium content of the slice at the start of the respective collection period and expressed as outflow (min^{-1}). For the evaluation of changes induced by the different treatments, a ratio was calculated by dividing the sum of fractional rates during the period of drug addition by that of the period without drug. Means $\pm$ S.E.M. are given throughout. Differences between means were tested for significance by the Mann–Whitney *U*-test.

2.3. Substances used in the experiments

5-[1,2-3*H*(*N*)]-Hydroxytryptamine creatinine sulphate, specific activity 26.7 Ci/mmol (NEN); fenfluramine (Servier); 6-nitroquipazine maleate (Tocris Cookson); pargyline HCl (Serva); reserpine (Sigma); tramadol HCl (Grünenthal). All substances were dissolved in water except reserpine which was dissolved by means of 1-methyl-2-pyrrolidone and then further diluted.

3. Results

3.1. Experiments with NaCl-containing superfusion medium

Prefrontal cortex slices, preincubated with 0.1 μM [^{3}H]5-HT, and then superfused with normal Krebs–Henseleit medium containing 10 μM pargyline, had a tritium content equivalent to 1.55 $\pm$ 0.07 pmol of [^{3}H]5-HT ($n = 31$) at the beginning of the collection periods, 50 min after onset of superfusion, as calculated from the sum of tritium contents in the slices and in the superfusates. During superfusion, the fractional rate of tritium outflow showed a steady decline (Fig. 1). After addition of the drugs, fractional rates of basal outflow rose as compared to controls; while the rise effected by reserpine and tramadol tended to reach a higher level paralleling that of controls, fenfluramine induced a steadily increasing enhancement of outflow during the observation period (Fig. 1). Evaluating the drug-induced basal outflow of tritium by comparing the sum of fractional rates after and before drug addition (Table 1), the rise induced by fenfluramine, 1 μM, was 78.1%, that by reserpine, 10 μM, 43.4% and that of tramadol, 10 μM, 54.9% and 100 μM, 84.0% above controls. The concentration of reserpine used was limited by its solubility.

Addition of 6-nitroquipazine, 1 μM, to the superfusion medium throughout superfusion enhanced the fractional rate of tritium outflow, by about 13% as determined in the collection period before drug addition, resulting in a lower tritium content in the slices at the start of the collection period, 0.91 $\pm$ 0.03 pmol of [^{3}H]5-HT equivalence. In addition, fractional rates declined more rapidly, resulting in a significant reduction in the ratio of fractional rates of tritium outflow under control conditions (Table 1). In the presence of nitroquipazine, there was no significant induction of basal outflow by fenfluramine, 1 μM, and a significant reduction of the facilitation produced by tramadol (at 100 μM 18.1% vs. 84.0%), while the stimulation of basal release induced by reserpine, 10 μM, appeared to be considerably enhanced (125.8% vs. 43.4%).

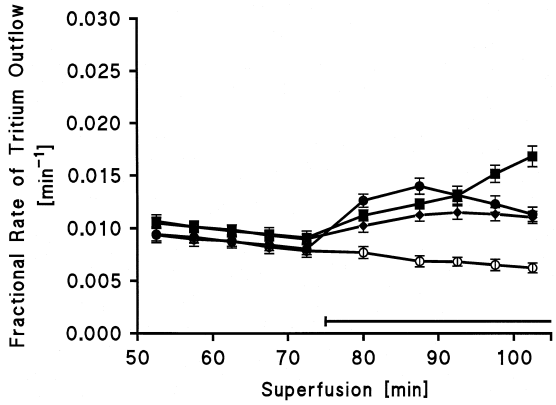

Fig. 1. Effect of fenfluramine, reserpine and tramadol on the basal outflow of tritium from prefrontal cortex slices preincubated with [^{3}H]5-HT. After preincubation, slices were superfused with medium containing 10 μM pargyline. Drugs were added after 75 min of superfusion (bar). (○) Controls, no drug added ($n = 6$); (■) fenfluramine, 1 μM ($n = 6$); (♦) reserpine, 10 μM ($n = 6$); (●) tramadol, 100 μM ($n = 6$).

Table 1
Effect of fenfluramine, reserpine and tramadol on the basal outflow of tritium from prefrontal cortex slices preincubated with [^{3}H]5-HT

Drug added after 75 min of superfusion	Medium containing throughout superfusion	Ratio of fractional rates after drug addition/before drug addition
Control		0.79 ± 0.01
Fenfluramine 1 μM	−	1.40 ± 0.04
Reserpine 10 μM	−	1.13 ± 0.01[a]
Tramadol 10 μM	−	1.22 ± 0.02[a]
Tramadol 100 μM	−	1.45 ± 0.04[a]
Control	Nitroquipazine 1 μM	0.73 ± 0.01[b]
Fenfluramine 1 μM	Nitroquipazine 1 μM	0.77 ± 0.01
Reserpine 10 μM	Nitroquipazine 1 μM	1.65 ± 0.07[a]
Tramadol 100 μM	Nitroquipazine 1 μM	0.86 ± 0.02[a]
Control	−NaCl; +LiCl	0.60 ± 0.02[b]
Fenfluramine 1 μM	−NaCl; +LiCl	0.67 ± 0.02[a]
Reserpine 10 μM	−NaCl; +LiCl	0.74 ± 0.02[a]
Tramadol 10 μM	−NaCl; +LiCl	0.57 ± 0.01
Tramadol 100 μM	−NaCl; +LiCl	0.63 ± 0.01
Control	−$CaCl_2$	0.74 ± 0.01[b]
Fenfluramine 1 μM	−$CaCl_2$	1.47 ± 0.07[a]
Reserpine 10 μM	−$CaCl_2$	1.10 ± 0.02[a]
Tramadol 10 μM	−$CaCl_2$	1.01 ± 0.02[a]
Tramadol 100 μM	−$CaCl_2$	1.22 ± 0.03[a]

After preincubation, slices were superfused with medium containing 10 μM pargyline. Nitroquipazine was added to, NaCl exchanged for LiCl, or $CaCl_2$ was omitted from the medium throughout superfusion, as indicated. Drugs were added after 75 min of superfusion. Means ± S.E.M. of 6–7 experiments. Significance of differences from corresponding controls; [a]$P < 0.05$. [b]Significantly different from controls without change of superfusion medium.

3.2. Experiments with medium containing LiCl instead of NaCl

Prefrontal cortex slices, preincubated with 0.1 μM [^{3}H]5-HT, and then superfused with medium containing 10 μM pargyline, in which NaCl had been replaced by LiCl, had a tritium content equivalent 0.43 ± 0.01 pmol of [^{3}H]5-HT ($n = 32$) at the beginning of the collection periods. During superfusion, the fractional rates of tritium outflow more than doubled compared to those observed in experiments with normal Krebs–Henseleit medium, and the decline of outflow was more pronounced (Fig. 2). After addition of the drugs, a small rise of outflow was induced by fenfluramine, 1 μM, by 13.2%, and a more pronounced effect was induced by reserpine, 10 μM, by 23.7% (Table 1). Tramadol did not change the basal release significantly.

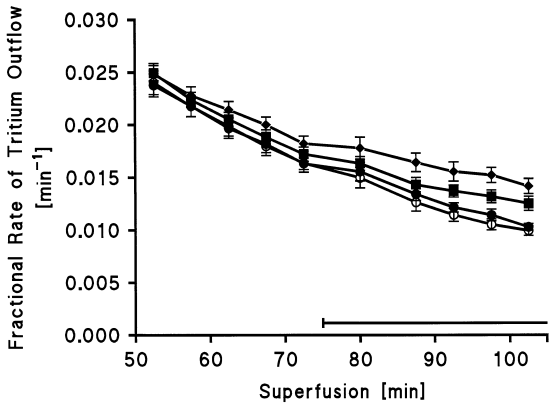

Fig. 2. Effect of fenfluramine, reserpine and tramadol on the basal outflow of tritium from prefrontal cortex slices preincubated with [^{3}H]5-HT. After preincubation, slices were superfused with medium containing 10 μM pargyline and LiCl instead of NaCl. Drugs were added after 75 min of superfusion (bar). (○) Controls, no drug added ($n = 7$); (■) fenfluramine, 1 μM ($n = 6$); (♦) reserpine, 10 μM ($n = 6$); (●) tramadol, 100 μM ($n = 6$).

3.3. Experiments with medium lacking $CaCl_2$

Omission of $CaCl_2$ from the superfusion medium did not affect the fractional rate of tritium outflow, as measured in the pre-drug period (not shown). However, the decline of basal outflow was accelerated, minimally reflected in the content of tritium in the slices at the onset of the collection periods, 1.47 ± 0.03 pmol of [^{3}H]5-HT equivalent, but shown by the lower ratio of fractional rates after and before 75 min of superfusion (Table 1). Without $CaCl_2$, the effect of fenfluramine, 1 μM, and of reserpine, 10 μM, on the basal outflow of tritium appeared somewhat enhanced compared to the condition with $CaCl_2$ added (99.5% vs. 78.1% and 50.0% vs. 43.3%, respectively), while the effect of tramadol, 10 and 100 μM, was considerably reduced, 36.8% vs. 54.9% and 65.9% vs. 84.0%, respectively. These differences in the tramadol groups reach statistical significance, however, since the ratios observed under control conditions (i.e., no drug added after 75 min) are already (slightly) different between

the two groups, this reduction must be looked at with caution.

4. Discussion

Studies on neurotransmitter release from nerve terminals are mainly performed using brain slices or isolated nerve terminals, synaptosomes. In superfusion studies, synaptosomes offer the advantage of less interaction between terminals, and once released neurotransmitter is not subject to reuptake (Raiteri and Levi, 1978), as observed in slices. Therefore, most studies demonstrating the existence of a carrier mediated outward transport of neurotransmitters, in contrast to exocytosis, have used synaptosomes (Levi and Raiteri, 1993). In slices, investigating the release of tritiated 5-HT, we could, however, distinguish between an enhancement of the basal release, probably carrier mediated release, and an enhanced stimulation-evoked overflow, which is effected by reuptake inhibition, when we compared the effects of 6-nitroquipazine and tramadol (Driessen and Reimann, 1992). Since tritiated 5-HT was used then and in the present investigation, and only tritium was determined without prior purification, pargyline was added to the experiments to avoid misinterpretation of the results obtained. The efficacy of pargyline to suppress metabolisation in release experiments has been shown in catecholamine release studies, and the concentration used in the present investigation has been shown, in our hands, to completely suppress deamination of [^{14}C]5-HT (Reimann and Schneider, 1993). Further implications of the use of pargyline in our experiments are discussed below.

In concordance with previously published studies, fenfluramine and reserpine (for overview: Levi and Raiteri, 1993) as well as tramadol (Driessen and Reimann, 1992) enhanced the outflow of 5-HT. The effects of fenfluramine were no longer observed, when a high concentration of the 5-HT uptake inhibitor 6-nitroquipazine was added to the superfusion medium, similar to results obtained with other uptake inhibitors like chlorimipramine (Maura et al., 1982) and fluoxetine (Gobbi et al., 1992), both in experiments with synaptosomal preparations. The antagonistic effects of uptake inhibitors was also observed in in vivo experiments in rats with microdialysis, where the release facilitating effect of fenfluramine was attenuated by citalopram (Kreiss et al., 1993) or fluoxetine and sertraline (Rutter and Auerbach, 1993). Reserpine enhances the cytosolic concentration of vesicularly stored monoamines by interference with the vesicular uptake carrier. As our results with 5-HT show does the resulting outflow from the neurones obviously not depend on the membrane carrier, since it is not blocked in the presence of a high concentration of 6-nitroquipazine. In contrast, the enhanced outflow in the presence of 6-nitroquipazine indicates, that under control conditions the uptake mechanism is considerably involved in the reduction of 5-HT loss. The enhancing effect of tramadol on the outflow of [^{3}H]5-HT is not quite abolished by the presence of the potent 5-HT uptake inhibitor 6-nitroquipazine. This may be due to the high concentration of tramadol used, however, the potency of 6-nitroquipazine is sufficient to block the quantitatively similar effect of fenfluramine. Therefore, it is conceivable that tramadol releases 5-HT by more than one mechanism.

Since the membrane carrier depends on the existence of a high concentration of extracellular Na^+ (overview: Lester et al., 1994), experiments were carried out under replacement of NaCl by an equimolar amount of LiCl, thereby creating a further condition for impairment of carrier mediated release. However, as can be depicted from the results under these conditions, there is an accelerated outflow of [^{3}H]5-HT coinciding with a rapid depletion. The induction of release by Na^+ depletion has also been suggested to depend on an activation of the membrane carrier (Maura et al., 1982), but studied on a synaptosomal preparation this was not supported, since outflow was not affected by immobilisers of the plasma membrane 5-HT carrier (Collard, 1989), confirming the 'knock-out' of this mechanism following Na^+ depletion. The enhanced release was tentatively assigned to an increase in membrane fluidity. Furthermore, addition of Li^+ induced a depolarisation in slices and synaptosomes (Adam-Vizi et al., 1987), thus probably activating exocytosis. So results obtained with slices which were rather depleted of 5-HT can only be supportive. Under these conditions, effects by fenfluramine are greatly reduced and those by tramadol virtually abolished. The most prominent effect was produced by reserpine, in contrast to the results obtained under control conditions. So these results are in agreement with the assumption of a membrane carrier involvement in the release induced by fenfluramine and tramadol, but not in the release induced by reserpine.

Ca^{2+} is inevitable and indicative for exocytosis. As expected, the release induced by reserpine was completely independent of external Ca^{2+}, thus, indicating its non-exocytotic nature. The effect of fenfluramine was also not reduced by the Ca^{2+}-removal. This result is somehow controversial to findings reported in the literature. While Gandhi and Jones (1990) found, that release induced by fenfluramine was Ca^{2+}-independent, others show a Ca^{2+}-dependency (Gobbi et al., 1992, 1993; Puig de Parada et al., 1995); the Ca^{2+}-channel involved has been reported to belong to the P-type (Frittoli et al., 1994). One difference between the studies demonstrating Ca^{2+}-independence, the present and that of Gandhi and Jones (1990), is the use of fenfluramine, while the other studies used D-fenfluramine. Another explanation may be assigned to the experimental conditions. Our results were obtained in the presence of a monoamine oxidase-inhibitor. As shown in neuronal cultures, monoamine oxidase-inhibition enhanced the intracellular content of 5-HT and subsequently the amount released by activation of the carrier (Gu and Azmitia, 1993). As incidenced by the results of Fitzgerald and Reid

(1993), this increase is mainly due to an enhanced cytoplasmic 5-HT concentration, a phenomenon also observed for noradrenaline after monoamine oxidase-blockade by pargyline (Trendelenburg, 1991). Therefore, it can be assumed that an enhanced cytoplasmic pool favours the carrier-mediated release, if a substance activates both, carrier mediated release and exocytosis.

The 5-HT release-enhancing effects of tramadol persisted without external Ca^{2+} in a dose-dependent manner. However, while the effects of fenfluramine and reserpine were quantitatively very similar, rather enhanced, compared to those observed in the presence of Ca^{2+}, those by tramadol were clearly reduced. Therefore, an exocytotic mechanism can be assumed to participate in the effect of tramadol. Whether this reflects a direct action on the nerve terminal, or an activation of an excitatory interneuron, which by release of an excitatory amino acid triggers exocytotic 5-HT release, requires further investigation.

Our results provide evidence that reserpine enhanced the release of 5-HT in a manner which does not involve the plasma membrane carrier or exocytosis. In comparison with the effects of reserpine, it can be assumed that tramadol does not interfere with the vesicular storage of 5-HT. Under our conditions, fenfluramine induced only membrane carrier mediated release of 5-HT. In addition, we found evidence that tramadol enhanced extraneuronal 5-HT concentrations not only by carrier mediated release of 5-HT, but also by induction of exocytosis.

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