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Short communication

Release studies with rat brain cortical synaptosomes indicate that tramadol is a 5-hydroxytryptamine uptake blocker and not a 5-hydroxytryptamine releaser

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Abstract

Tramadol is a centrally acting opioid analgesic whose mechanism of action could also involve an increase in central serotoninergic transmission. Thus, tramadol inhibits synaptosomal serotonin (5-hydroxytryptamine, 5-HT) reuptake and induces tritium release from [^{3}H]5-HT-preloaded slices. We investigated the effect of (±)-tramadol in release studies with superfused rat brain cortex synaptosomes preloaded with [^{3}H]5-HT. Tramadol had no releasing effect up to 30 μM, whereas at 10 μM tramadol significantly inhibited by 45% D-fenfluramine-induced [^{3}H]5-HT release. At 100 μM, tramadol showed a slight releasing effect in the absence or in the presence of pargyline, which was not augmented in synaptosomes pre-exposed to Ro 04-1284 (2-ethyl-1,3,4,6,7,11b-hexahydro-3-isobutyl-9,10-dimethoxy-2*H*-benzo [a]quinolizin-2-ol hydrochloride), a reserpine-like compound that enhances cytoplasmic 5-HT levels. In summary, (±)-tramadol behaved as a classical 5-HT uptake blocker (like citalopram) and not as a substrate of the 5-HT carrier with indirect 5-HT mimetic properties (like D-fenfluramine). © 1999 Elsevier Science B.V. All rights reserved.

Keywords: (±)-Tramadol; 5-HT (5-hydroxytryptamine, serotonin) carrier; 5-HT (5-hydroxytryptamine, serotonin) release; Synaptosome

1. Introduction

Tramadol is a centrally acting opioid analgesic with affinity for μ-opioid receptors in the low micromolar range (Hennies et al., 1988; Raffa et al., 1992). It was, however, suggested that an increase in monoaminergic transmission might also contribute to its antinociceptive effects (Driessen and Reimann, 1992; Raffa et al., 1992; Giusti et al., 1997). Thus, it was shown that tramadol inhibited the uptake of serotonin (5-hydroxytryptamine, 5-HT) and noradrenaline (but not dopamine) into rat brain synaptosomes in the low micromolar range (Driessen and Reimann, 1992; Raffa et al., 1992; Driessen et al., 1993; Giusti et al., 1997). In release studies with [^{3}H]noradrenaline-preloaded slices, tramadol (mainly the (−) enantiomer) mimicked the effects of cocaine, suggesting that it is a classical noradrenaline uptake blocker (Driessen et al., 1993). However, similar studies with [^{3}H]5-HT-preloaded slices suggested that tramadol (mainly the (+) enantiomer) is a substrate of the 5-HT carrier with 5-HT-releasing activity, thus resembling an indirect 5-HT mimetic agent (like d-fenfluramine) more than a classical 5-HT uptake blocker (Driessen and Reimann, 1992; Reimann and Schneider, 1998). In particular, these authors found that tramadol was active in inducing tritium release at concentrations as low as 1–10 μM and that this effect was antagonized by 6-nitro-quipazine, a well-known 5-HT uptake blocker.

In the present study, we reconsidered this proposed mechanism by studying the effects of (±)-tramadol in release studies with superfused [^{3}H]5-HT-preloaded synaptosomes. With this experimental approach, and differently from [^{3}H]5-HT-preloaded slices, the neurotransmitter released by the drug is immediately removed and cannot be retaken up (Raiteri et al., 1974; Gobbi et al., 1992). Thus, the releasing effect is completely distinct from uptake inhibition. This is particularly important when studying the effects of those compounds that are believed to act both as releasers and reuptake inhibitors (Crespi et al., 1997). Moreover, the previous studies were carried out in the presence of pargyline (Driessen and Reimann, 1992; Reimann and Schneider, 1998), which is likely to increase

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the cytoplasmic 5-HT concentration (Fitzgerald and Reid, 1993; Reimann and Schneider, 1998). In order to have data more related to the physiological situation, we carried out these experiments in the absence of the monoamine–oxidase inhibitor, so that 5-HT was probably mainly stored in synaptic vesicles.

2. Materials and methods

2.1. Experimental procedure

Male CRL:CD(SD)BR rats (Charles River, Italy) weighing about 150 g were used. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., suppl. 40, Feb. 18, 1992) and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, Dec.12, 1987; Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996).

The rats were killed by decapitation and their brain cortices were rapidly dissected and homogenized in 40 volumes of ice-chilled 0.32 M sucrose, pH 7.4, in a glass homogenizer with a Teflon pestle. The homogenates were centrifuged at $1000 \times g$ for 5 min and the supernatants were centrifuged again at $12\,000 \times g$ for 20 min to yield the crude synaptosomal pellet (P_2) (Gray and Whittaker, 1962).

For the release studies, the P_2 pellets were resuspended in about 20 vol. of Krebs–Henseleit buffer of the following composition (mM): NaCl (125); KCl (3); $CaCl_2$ (1.2); $MgSO_4$ (1.2); NaH_2PO_4 (1); $NaHCO_3$ (22); glucose (10), gassed with 95% O_2 and 5% CO_2, pH 7.2-7.4. The suspension was then added to an equal volume of the same buffer containing [^{3}H]5-HT (S.A. 29.7 Ci/mmol) at the final concentration of 0.06 μM. After a 15-min incubation at 37°C, the solution was diluted with fresh buffer and 5-ml samples (about 5 mg initial tissue) were distributed onto cellulose mixed ester filters (0.65-μm pore size, Millipore) in a 16-chamber superfusion apparatus thermostatically held at 37°C (Raiteri et al., 1974).

The synaptosomes were layered onto the filters by aspiration from the bottom under moderate vacuum. Superfusion was started ($t = 0$ min) at a rate of 0.5 ml/min with standard medium; after a 42-min equilibration period, fractions were collected every 2 min until t = 60 min. The filters and the fractions were put into scintillation vials and counted for radioactivity in 4 ml of Ultima Gold MV (Packard).

(±)-Tramadol and D-fenfluramine were present in the superfusion medium for 3 min from $t = 47$ to $t = 50$ min. In some experiments their releasing properties were evaluated in synaptosomes pre-exposed (from to = 40) to Ro 04-1284. In other experiments the releasing effect of D-fenfluramine was measured in synaptosomes which had been pre-exposed (from $t = 40$) to tramadol or citalopram. When it was used, pargyline (250 μM) was present during preloading and throughout the superfusion period.

The fractional release rate was calculated as 100 times the amount of radioactivity released into each 2-min fraction divided by the total radioactivity present on the filter at the start of that fraction. The fractional release rates before the releasing stimulus was applied (t = 44-46), expressed as percentage, are reported as basal outflow. The overflow (%) was calculated as the difference between the fractional release rates in the presence ($t = 48$–56) and absence of the drug (mean $t = 44$–48 and $t = 56$–60) (see Fig. 1). When synaptosomes were preexposed to Ro 04-1284 (from $t = 40$), the drug-induced overflow was calculated by subtracting the fractional release rate in the absence of the drug from the fractional release rate in the presence of the drug (see Fig. 2). The effect of the drugs added at $t = 47$ could be detected only 1 min later because the fluid took about 1.5 min to flow from the filters to the collecting vials.

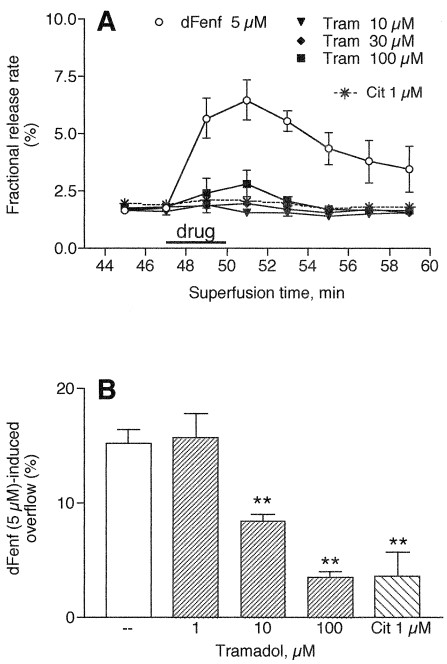

Fig. 1. Tritium release from superfused rat cortical synaptosomes preloaded with [^{3}H]5-HT. These experiments were carried out in the absence of pargyline. (A) Concentration–response curve for tritium release induced by (±)-tramadol (tram) and comparison with the release induced by 5 μM d-fenfluramine (dFenf) and the lack of releasing effect of 1 μM citalopram (cit). Each value is the mean ± S.E. of three different experiments. (B) Effect of (±)-tramadol in inhibiting the [^{3}H]5-HT release induced by 5 μM D-fenfluramine. The inhibitory effect of 1 μM citalopram (cit) is shown for comparison. In this experiment, (±)-tramadol and citalopram were present from $t = 40$ throughout the experiment, whereas D-fenfluramine was added from $t = 47$ to $t = 50$ min. Each value is the mean ± S.E. ($n = 4$ replications). $**$ $P < 0.01$ different from control value (Student's t-test).

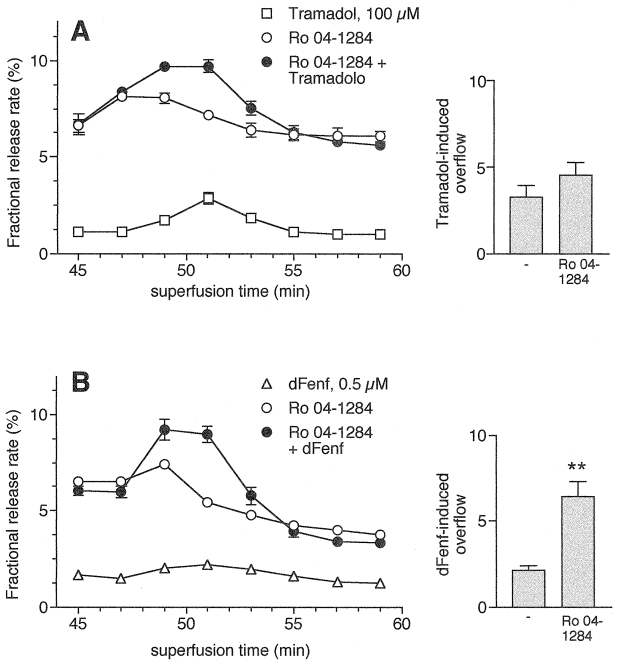

Fig. 2. Effect of (±)-tramadol (100 μM) (A) and D-fenfluramine (0.5 μM) (B) on superfused rat brain cortex synaptosomes preloaded with [^{3}H]5-HT, in the absence or the presence of 0.1 μM Ro 04-1284. Ro 04-1284 was present in the superfusion medium from $t = 40$ whereas tramadol and D-fenfluramine were added from $t = 47$ to $t = 50$. These experiments were carried out in the presence of pargyline. For the experiment with D-fenfluramine only (B) a Ca^{2+}-free medium was used from $t = 40$ throughout superfusion. Drug ((±)-tramadol or D-fenfluramine)-induced overflow in the absence or presence of Ro 04-1284 (right panels) was calculated as described in Section 2. Each value is the mean ± S.E. of 4 replications from a single experiment. $**P < 0.01$ different from the value in the absence of Ro 04-1284 (Student's *t*-test). These experiments were replicated twice with similar results and identical results for the statistical analysis.

2.2. Substances used in the experiments

(±)-Tramadol HCl was purchased as an injectable solution (Contramal®, Prodotti Formenti, Milano, Italy). D-Fenfluramine HCl was from Servier (Orleans, France) and Ro 04-1284 (2-ethyl-1,3,4,6,7,11b-hexahydro-3-isobutyl-9,10-dimethoxy-2*H*-benzo [a]quinolizin-2-ol hydrochloride) was from Hoffmann-La Roche (Basel, Switzerland). [^{3}H]5-HT was purchased from Amersham (Buckinghamshire, UK).

3. Results

In the absence of pargyline, (±)-tramadol showed no releasing activity up to 30 μM while at 100 μM it induced a very low release of tritium (2.2 ± 1.4%, mean ± S.E., $n = 3$ experiments), much lower than the release induced by 5 μM D-fenfluramine (15.2 ± 1.2%) (Fig. 1A). The classical 5-HT uptake blocker citalopram, tested at a concentration (1 μM) which should completely inhibit 5-HT transport, had no releasing activity (Fig. 1A), thus confirming that, with the superfusion apparatus used the released neurotransmitter is immediately removed and cannot be retaken up (Raiteri et al., 1974; Gobbi et al., 1992). Under the same conditions, 1 μM citalopram antagonized d-fenfluramine-induced tritium release (77% inhibition at 1 μM, Fig. 1B), which is known to be 5-HT carrier-mediated (Levi and Raiteri, 1993; Crespi et al., 1997). The releasing effect of D-fenfluramine was inhibited by (±)-tramadol too (45% and 77% inhibition at 10 and 100 μM, Fig. 1B).

In order to verify whether the reported releasing effects of tramadol would require an increased concentration of cytoplasmic 5-HT, we first repeated the experiment in the presence of pargyline, as in previous studies (Driessen and Reimann, 1992; Reimann and Schneider, 1998). In the presence of 250 μM pargyline, however, 100 μM (±)-tramadol still had a very low releasing effect (tritium overflow of 2.6 ± 0.6%, mean ± S.E., $n = 8$ replications from 2 experiments). We also evaluated the releasing effect of (±)-tramadol in synaptosomes pre-exposed to 0.1 μM Ro 04-1284, a vesicular monoamine transporter type-II inhibitor which rapidly depletes secretory vesicles (Colzi et al., 1993), thus increasing the cytoplasmic 5-HT concentration (in the presence of 250 μM pargyline), as confirmed by the increased [^{3}H]5-HT outflow (Fig. 2A,B). Under this condition, (±)-tramadol also had a low releasing effect (Fig. 2A). As expected, under these conditions, the carrier-mediated releasing effect of 0.5 μM D-fenfluramine significantly increased (Fig. 2B). This experiment with D-fenfluramine was carried out in the absence of Ca^{2+} in order to avoid exocytotic-like release (Gobbi et al., 1992; Crespi et al., 1997). At this low concentration (0.5 μM) and in the absence of Ca^{2+}, D-fenfluramine-induced release was very low, which indicates a very limited effect of the drug on the synaptic vesicles.

4. Discussion

It was previously described that (±)-tramadol inhibits synaptosomal [^{3}H]5-HT uptake in the low micromolar range (1-3 μM) (Driessen and Reimann, 1992; Raffa et al., 1992; Driessen et al., 1993; Giusti et al., 1997). This effect could be due to a citalopram-like effect (i.e., an action as a pure 5-HT uptake blocker) or to a d-fenfluramine-like effect (i.e., an action as a 5-HT carrier substrate). The present data, obtained using [^{3}H]5-HT preloaded synaptosomes, support the former possibility. Thus, 10 μM (±)-tramadol significantly inhibited the carrier-mediated d-fenfluramine-induced [^{3}H]5-HT release, as did citalopram, but it did not mimic the [^{3}H]5-HT releasing effect of d-fenfluramine at concentrations up to 30 μM. These experiments were carried out in the absence of pargyline, i.e., in the absence of cytoplasmic 5-HT. However, we did not measure the 5-HT-releasing effect of (±)-tramadol

under experimental conditions which increase the cytoplasmic 5-HT concentration, i.e., in synaptosomes pre-exposed to Ro 04-1284, a reserpine-like compound which rapidly depletes secretory vesicles (Colzi et al., 1993). Under this experimental condition, it is expected that a substrate of the carrier favours carrier-dependent 5-HT release, according to the phenomenon of 'accelerated exchange diffusion' (Trendelemburg, 1979). These data suggest that tramadol is not a substrate of the 5-HT carrier and further confirm the view that it is a classical 5-HT uptake blocker, in agreement with data showing that in vivo tramadol has the same influence on 5-HT turnover as fluoxetine (Frink et al., 1996). We have no clear explanation for the discrepancy with previous data (Driessen and Reimann, 1992; Reimann and Schneider, 1998) obtained using [^{3}H]5-HT-preloaded slices and showing a D-fenfluramine-like 5-HT-releasing effect of tramadol. Possibly, the differences in experimental models (slices vs. synaptosomes) could have contributed to the different results.

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References

Colzi, A., D'Agostini, F., Cesura, A.M., Da Prada, M., 1993. Monoamine oxidase-A inhibitors and dopamine metabolism in rat caudatus: evidence that an increased cytosolic level of dopamine displaces reversible monoamine oxidase-A inhibitors in vivo. J. Pharmacol. Exp. Ther. 265, 103–111.

Crespi, D., Mennini, T., Gobbi, M., 1997. d-Amphetamine and related compounds (3,4-methylendioxymethamphetamine, *p*-chloroamphetamine and D-fenfluramine) release [3H]5-HT and/or [3H]DA from rat brain synaptosomes with both a carrier-dependent and a Ca2+-dependent mechanism. Br. J. Pharmacol. 121, 1735–1743.

Driessen, B., Reimann, W., 1992. Interaction of the central analgesic, tramadol, with the uptake and release of 5-hydroxytryptamine in the rat brain in vitro. Br. J. Pharmacol. 105, 147–151.

Driessen, B., Reimann, W., Giertz, H., 1993. Effects of the central analgesic tramadol on the uptake and release of noradrenaline and dopamine in vitro. Br. J. Pharmacol. 108, 806–811.

Fitzgerald, J.L., Reid, J.J., 1993. Interactions of methylenedioxymethamphetamine with monoamine transmitter release mechanisms in rat brain slices. Naunyn-Schmiedeberg's Arch. Pharmacol. 347, 313–323.

Frink, M.C., Hennies, H.H., Englberger, W., Haurand, M., Wilffert, B., 1996. Influence of tramadol on neurotransmitter system of the rat brain. Arzneim. Forschung. 46, 1029–1036.

Giusti, P., Buriani, A., Cima, L., Lipartiti, M., 1997. Effect of acute and chronic tramadol on [3H]-5-HT uptake in rat cortical synaptosomes. Br. J. Pharmacol. 122, 302–306.

Gobbi, M., Frittoli, E., Mennini, T., Garattini, S., 1992. Releasing activities of D-fenfluramine and fluoxetine on rat hippocampal synaptosomes preloaded with [^{3}H]serotonin. Naunyn-Schmiedeberg's Arch. Pharmacol. 345, 1–6.

Gray, E.G., Whittaker, V.P., 1962. The isolation of nerve endings from brain: an electron-microscope study of cell fragments derived by homogenization and centrifugation. J. Anat. 96, 79–87.

Hennies, H.-H., Friderichs, E., Schneider, J., 1988. Receptor binding, analgesic and antitussive potency of tramadol and other selected opioids. Arzneim. Forschung./Drug Res. 38, 877–880.

Levi, G., Raiteri, M., 1993. Carrier-mediated release of neurotransmitters. Trends Neurosci. 16, 415–419.

Raffa, R.B., Friderichs, E., Reimann, W., Shank, R.P., Codd, E.E., 1992. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic. J. Pharmacol. Exp. Ther. 260, 275–285.

Raiteri, M., Angelini, F., Levi, G., 1974. A simple apparatus for studying the release of neurotransmitters from synaptosomes. Eur. J. Pharmacol. 25, 411–414.

Reimann, W., Schneider, F., 1998. Induction of 5-hydroxytryptamine release by tramadol, fenfluramine and reserpine. Eur. J. Pharmacol. 349, 199–203.

Trendelemburg, U., 1979. Release induced by phenethylamines. In: Paton, D.M. (Ed.), The Release of Catecholamines from Adrenergic Neurons. Pergamon, Oxford, pp. 333–354.