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Effect of the opioid analgesic tramadol* on inactivation of norepinephrine and serotonin

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Interference with norepinephrine (NE)- and serotonin (5-HT)-inactivation by blockade of neuronal uptake is a common property of many opioid analgesics and antagonists [3–5, 11–13, 16, 18, 21]. Interference of opioid-like analgesics with amine inactivation may be clinically relevant as known from severe reactions due to MAO inhibitors and potentiation of NE pressor response in pethidine pretreated patients [8, 20].

Tramadol is a new synthetic opioid with analgesic potency comparable to pentazocine, codeine and dextropropoxyphene [1, 6]. It increased the NE pressor response in anaesthetized cats [14] and pithed rats [7]. Further, tramadol toxicity was enhanced in tranylcypromine-pretreated mice [7]. A possible interference of tramadol with monoamine inactivation was therefore investigated.

Materials and methods

Methods. LD_{50} Values, 24 hr after intraperitoneal application of test drugs were determined in groups of 10 male mice (NMRI, breeder Schmitz, 18–25 g). In parallel, the animals were pretreated with 10 mg/kg i.p. tranylcypromine 2 hr before application of test drugs and distance factors of lethality curves were calculated according to Litchfield and Wilcoxon [9].

Synaptosomal uptake of monoamines was determined according to Raiteri *et al.* [15] with several modifications as follows: Krebs–Ringer medium pH 7.3 (128 mM NaCl, 2.7 mM $CaCl_2$, 1.2 mM $MgSO_4$, 5 mM KCl, 5 mM Na_2HPO_4, 10 mM Tris–HCl); synaptosomal P_2 fraction from whole rat brain prepared by the method of Whittaker [22] (approx. 300 μg protein per assay, protein determination according to Lowry *et al.* [10]; iproniazid (12.5 μM); and ascorbic acid (1 mM) were preincubated in an atmosphere of 95% O_2 + 5% CO_2 for 5 min at 30°. Then, the uptake inhibitor 10^{-6}–10^{-3} M), radioactive substrate ([^{3}H]NE or [5-^{3}H]HT: 1×10^{-7} M, 0.5 μCi), and buffer to give a final volume of 1.1 ml, were added. After 2 min at 30°, uptake was stopped by cooling the tubes in ice. Two 500 μl aliquots per sample were filtered through cellulose–nitrate filters (Schleicher and Schüll; BA 85; Ø 15 mm; 0.45 μm pore size). The filters were rinsed with 10 ml of 0.32 M sucrose, solubilized in 500 μl cellosolve, mixed with 10 ml of a liquid scintillation solution and counted. Control values for [5-^{3}H]HT and [^{3}H]NE uptake rates were: 44,443 ± 2578 dpm × mg^{-1} per min (n = 26) and 14,789 ± 610 dpm × mg^{-1} per min (n = 24), respectively. Data are reported as means ± S.E.M., and significance was determined by the two tailed Student's t-test.

The influence of test drugs on monoamine oxidase (MAO) [14] isolated from rat liver mitochondria was determined according to Snyder and Hendley [19] with benzylamine, octopamine and tyramine as substrates.

Catechol-*O*-methyltransferase (COMT) was partially purified according to Axelrod [2] from rat liver (sp. act. 5.6 mU/mg protein) and stored in the presence of 2 mM DTE at −20° (one unit of enzyme activity is defined as that amount which causes the synthesis of 1 μmole of metanephrine from epinephrine in 1 min). The enzyme activity was determined according to Schwabe and Flohé [17].

Chemicals and drugs. DL-[7-^{3}H(N)]norepinephrine (5–15 Ci/mmole), New England Nuclear (Boston, MA) (diluted with L-NE before use); 5-[1,2-^{3}H(N)]hydroxytryptamine binoxalate (15–30 Ci/mmole), New England Nuclear; *S*-adenosyl-L-[methyl-^{3}H]methionine (100–500 mCi/mmole), Amersham Buchler (Braunschweig, West Germany); Tramadol–HCl, Grünenthal GmbH (Aachen, West Germany); pethidine–HCl, Hoechst AG (Frankfurt, West Germany); morphine–HCl, Merck (Darmstadt, West Germany); cocaine–HCl, Merck; L-methadone–HCl, Hoechst AG; imipramine–HCl, Ciba-Geigy (Basel, Switzerland); tranylcypromine–HCl, Röhm

* Tramal®, Grünenthal GmbH, Aachen, West Germany.

Table 1. *In vitro* inhibition of monoamine uptake and effect of tranylcypromine pretreatment on acute toxicity of tramadol and reference compounds.

Substance	LD_{50} Value (mg/kg i.p.) Without pretreatment	With tranylcypromine pretreatment	Distance factor of lethality curves	Inhibition of [5-^{3}H]HT uptake, IC_{50} (M)	Inhibition of [^{3}H]NE uptake, IC_{50} (M)
Tramadol	166 (157–175)	90.8 (81.1–102)	1.8 (1.6–2.1)	4.05×10^{-5} (2.25–7.17×10^{-5})	1.38×10^{-5} (0.91–2.12×10^{-5})
L-Methadone	37.2 (31.6–43.8)	19.8 (14.4–27.1)	1.9 (1.3–2.7)	4.22×10^{-6} (1.66–8.33×10^{-6})	3.23×10^{-6} (1.37–5.99×10^{-6})
Pethidine	121 (109–135)	64.3 (47.5–87.2)	1.9 (1.4–2.6)	3.73×10^{-6} (1.22–8.02×10^{-6})	2.83×10^{-6} (1.13–5.37×10^{-6})
Morphine	432 (341–547)	109 (70.9–167)	4.0 (2.4–6.5)	$>5 \times 10^{-4}$	$>5 \times 10^{-4}$
Imipramine	99.5 (78.4–126)	64.6 (51.6–80.9)	1.5 (1.1–2.1)	2.89×10^{-6} (1.19–5.37×10^{-6})	3.92×10^{-6} (2.54–5.65×10^{-6})
Cocaine	74.5 (65–85.3)	59.4 (53.1–66.6)	1.3 (1.1–1.5)		6.85×10^{-8} (4.27–10.36×10^{-8})

Confidence limits are shown in parentheses.

Pharma (Darmstadt, West Germany); iproniazid phosphate, Forestex (The Haag, The Netherlands); L-norepinephrine-bitartrate, Serva (Heidelberg, West Germany); L-epinephrine, Serva.

Results and discussion

Tranylcypromine substantially increases the toxicity of all compounds investigated (Table 1). The most prominent increase was found with morphine, lethality is about 4-fold higher than without pretreatment. In combination with tranylcypromine the lethality of methadone, pethidine and tramadol is about 2-fold higher, whereas toxicity of imipramine and cocaine is only slightly, but nevertheless significantly, increased.

In rat brain synaptosomes tramadol inhibits [5-^{3}H]HT uptake with an IC_{50} value of 4.05×10^{-5} M thus showing a potency approximately one order of magnitude lower than methadone, pethidine and imipramine. With an IC_{50} value of $>5 \times 10^{-4}$ M, morphine is only a very weak 5-HT uptake inhibitor (Table 1). Tramadol, methadone, pethidine, morphine and imipramine inhibit synaptosomal [^{3}H]NE uptake in concentration ranges similar to those affecting 5-HT uptake. With an IC_{50} value of 6.85×10^{-8} M cocaine is the strongest [^{3}H]NE uptake blocker tested (Table 1). Tramadol does not have any influence on the activities of mitochondrial rat liver MAO-A and MAO-B and soluble rat liver COMT up to a concentration of 10^{-4} M (data not shown).

An increase of biogenic amines, particularly of 5-HT is believed to be responsible for toxic interactions of MAO-inhibitors with opioid analgesics [16]. As shown by many investigators [4, 5, 11, 12, 21] synaptosomal 5-HT (as well as NE uptake) is inhibited by several opioid analgesics. Therefore, uptake inhibition in combination with impaired metabolic inactivation by blockade of MAO might increase the cerebral monoamine content to a toxic level [16]. However, comparison of the potentiation of acute toxicity in mice by MAO-inhibitor pretreatment (Table 1) with the inhibition of synaptosomal 5-HT uptake *in vitro* reveals no quantitative correlation: morphine only slightly inhibited the 5-HT uptake *in vitro* but its toxicity was increased 4-fold by tranylcypromine. MAO inhibition increased the toxicity of tramadol, pethidine and methadone 2-fold, while the 5-HT uptake blocking potency of tramadol was only approximately 1/10 of pethidine and methadone. Toxicity of the uptake blocker imipramine was only slightly affected (1.5-fold increase).

The inhibition of NE uptake by the opioids investigated does not correlate with the increased toxicity induced by MAO inhibitors either. Certainly, monoamine uptake blockade may contribute to the toxicity of opioids in animals treated with MAO inhibitors. Considering the quantitative correlations, however, additional, yet unclarified interferences must be involved.

In principle, NE uptake blockade by tramadol implicates the risk of an enhanced pressor response to endogenous or exogenous NE. The blood levels obtained after recommended therapeutic dosages of tramadol in humans, however, ($<3\ \mu$M, [7]) range below the concentrations effectively inhibiting NE uptake in rat synaptosomes *in vitro*. Correspondingly, a potentiation of endogenous NE effects by tramadol has not been observed so far. Nevertheless, blood pressure effects merit special attention, if regular dosages of tramadol are exceeded for some reason, and combined therapy with other drugs impairing monoamine-inactivation should be avoided.

In conclusion, tramadol does not affect metabolic inactivation of monoamines, but impairs uptake of NE and 5-HT in brain synaptosomes, although to a lesser extent than the other opiate agonists investigated with the exception of morphine. Although pertinent clinical complications are hardly predictable and have so far only been established for pethidine, adequate precautions are recommended.

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