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THE NON-PEPTIDIC DELTA OPIOID RECEPTOR AGONIST TAN-67 ENHANCES DOPAMINE EFFLUX IN THE NUCLEUS ACCUMBENS OF FREELY MOVING RATS VIA A MECHANISM THAT INVOLVES BOTH GLUTAMATE AND FREE RADICALS

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Abstract—The activation of the δ-opioid receptors in the nucleus accumbens is known to induce a large and rapid increase of accumbal dopamine efflux. (±)-TAN-67 (2-methyl-4aα-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12aα-octahydro-quinolino[2,3,3,-g]isoquinoline) is a centrally acting non-peptidic δ opioid receptor agent which has recently become available. Interestingly, the (+) enantiomer of TAN-67 induces hyperalgesia in contrast to the (−) enantiomer of TAN-67 that produces profound antinociceptive effects in mice; the latter effects are mediated through δ-1 receptor stimulation. Using the microdialysis technique, the ability of the enantiomers of TAN-67 to alter the release of accumbal dopamine *in vivo* was analyzed.

Like the 25-min infusion of the selective δ-1 opioid receptor agonist (D-[Pen^{2,5}]-enkephalin) DPDPE (50 nM) and the δ-2 opioid receptor agonist deltorphin II (50 nM), the 25-min infusion of both (−)-TAN-67 (25 and 50 nM) and (+)-TAN-67 (25 and 50 nM) into the nucleus accumbens produced a similar transient dose-dependent increase in the accumbal extracellular dopamine level. Naloxone (1 mg/kg i.p., given 25 min prior to the drugs), namely a treatment that is known to inhibit the increase of dopamine induced by DPDPE and deltorphin II, did not affect the transient increase in the accumbal dopamine level produced by infusion of the enantiomers of TAN-67. The DPDPE and deltorphin II-induced increase in accumbal dopamine level, but not that of (−)-TAN-67 and (+)-TAN-67, was eliminated by subsequently perfused tetrodotoxin (2 μM) into the nucleus accumbens. The increase in accumbal dopamine level produced by an infusion of (−)-TAN-67 and (+)-TAN-67 was not altered by a Ca^{2+}-free Ringer's solution. The (−)-TAN-67 and (+)-TAN-67-induced accumbal dopamine efflux was strongly prevented by reserpine (5 mg/kg i.p., given 24 h earlier) or α-methyl-para-tyrosine (250 mg/kg i.p., given 2 h earlier). The effects of the enantiomers of TAN-67 on the accumbal dopamine were nullified by combined treatment with reserpine and α-methyl-para-tyrosine. The (−)-TAN-induced dopamine efflux was significantly reduced by the *N*-methyl-D-aspartate (NMDA) receptor antagonists ifenprodil (20 mg/kg i.p., 20 min before) and MK-801 (0.5 mg/kg i.p., 20 min before), respectively. The effects of (−)-TAN-67 on the dopamine efflux were also inhibited by the free radical scavenger *N*-2-mercaptopropionyl glycine (100 mg/kg i.p., 20 min before).

These results show that both enantiomers of TAN-67 enhance the release of reserpine sensitive, vesicular dopamine and α-methyl-*p*-tyrosine sensitive, cytosolic dopamine from dopaminergic nerve terminals in the nucleus accumbens in a way that is independent of neural activity; activation of δ opioid receptors plays no role in these events. All together, the results suggest that (−)-TAN-67 can generate a burst of free radicals that in turn trigger a release of glutamate that ultimately via activation of NMDA receptors enhances the release of dopamine from dopaminergic nerve terminals in the nucleus accumbens.

Key words: TAN-67, dopamine, nucleus accumbens, microdialysis, glutamate, free radical.

(±)-TAN-67(2-methyl-4aα-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12aα-octahydro-quinolino[2,3,3,-g]isoquinoline) is a centrally acting non-peptidic δ opioid receptor agent which has recently become available (Nagase et al., 1998, 2001). Despite its highly agonistic activity *in vitro* (Nagase et al., 1998, 2001), (±)-TAN-67 has weak or no antinoceptive action *in vivo* (Kamei et al., 1995; Suzuki et al., 1995). Interestingly, the (+) enantiomer of TAN-67 induces hyperalgesia (Tseng et al., 1997) in contrast to the (−) enantiomer of TAN-67 that produces profound antinociceptive effects in mice; the latter effects are mediated through δ-1 receptor stimulation (Tseng et al., 1997; Kamei et al., 1997). The nucleus accumbens (NAc) contains various subtypes of opiate receptor: μ-, κ-, and δ-opioid receptors. Stimulation of the μ- or δ-opioid receptor subtypes in the

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Abbreviations: ANOVA, analysis of variance; deltorphin-II, [D-Ala^2,Glu^4]-deltorphin; DPDPE, D-[Pen^{2,5}]-enkephalin; EDTA, ethylendiaminetetraacetic acid; α-methyl-para-tyrosine, α-methyl-para-tyrosine methyl ester; MK-801, (+)-5-methyl-10,11-dihydro-5H-dibenzo [a, b]cyclohepten-5,10-imine; N-2-MPG, *N*-2-mercaptopropionyl glycine; NAc, nucleus accumbens; NMDA, *N*-methyl-D-aspartate; reserpine, (methyl 18β-(3,4,5-trimethoxybenzoyl) oxy-11, 17α-dimethoxy-3β, 20α-yohimban-16β-carboxylate; TAN-67, 2-methyl-4a α-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12a α-octahydro-quinolino-[2,3,3-g]isoquinoline; TTX, tetrodotoxin.

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NAc induces a large and rapid increase of accumbal dopamine efflux. Thus, fentanyl, a μ receptor agonist, DPDPE, a δ-1 receptor agonist (Yoshida et al., 1999), and deltorphin-II, a δ-2 receptor agonist (Longoni et al., 1991), can increase the accumbal dopamine efflux. The relationship between the antinociceptive action of opioid receptor agonists and the release of dopamine in the NAc is unknown. Given the differential effects of (−) and (+) TAN-67 upon nociception, we analyzed the effects of (−)-TAN-67 and (+)-TAN-67 on the release of dopamine in the NAc, using the microdialysis technique that we have assessed in our previous studies (Murai et al., 1994; Takada et al., 1993; Tomiyama et al., 1993, 1995; Fusa et al., 2002). Second, the effects of naloxone on the (−)-TAN-67 or (+)-TAN-67-induced dopamine efflux were investigated, since naloxone acts as an antagonist at all opioid receptor subtypes, including the δ opioid receptor (Heijna et al., 1990; Yilmaz et al., 1998). Because we found that the drug-induced increase of the extracellular amount of accumbal dopamine was not at all attenuated by naloxone, the question arose whether the drug-induced efflux of accumbal dopamine was dependent on neuronal activity. For that reason, we examined the effects of the sodium channel blocker tetrodotoxin (TTX) and Ca^{2+}-free ringer on extracellular dopamine concentrations. For comparison, the effects of TTX on the accumbal dopamine efflux induced by other δ receptor agonists such as DPDPE and deltorphin-II were studied.

Given the finding that the drug-induced efflux of accumbal dopamine was independent of the neuronal activity, the question arose whether the dopamine had a neuronal origin. Therefore, we examined to what extent the vesicular and/or cytosolic pools in dopaminergic nerve terminals contributed to the drug-induced efflux of accumbal dopamine. The contribution of the vesicular pool of dopamine was assessed in experiments using reserpine, a compound that is known to inhibit the vesicular monoamine transporter (Peter et al., 1994) and, accordingly, to deplete vesicular dopamine, whereas the contribution of the cytosolic pool of dopamine was assessed in experiments using α-methyl-para-tyrosine, an agent that is known to inhibit the synthesis of dopamine in the cytosol (Besson et al., 1969; Groppetti et al., 1977; Javoy and Glowinski, 1971).

Since we found that the TAN-67-induced efflux of accumbal dopamine originated from both the vesicular and the cytosolic pools, the ultimate question arose whether the drug-induced increase of the extracellular amount of accumbal dopamine was due to stimulation of presynaptic receptors on dopaminergic nerve terminals. An important candidate receptor was the glutamate receptor, because glutamate has been found to facilitate the release of dopamine (Leviel et al., 1990; Krebs et al., 1991; Desce et al., 1992; Lonart and Zigmond, 1991) and glutamate antagonists have been found to inhibit the release of dopamine (Remblier et al., 1999), although this is not a consistent finding (Mathe et al., 1999). Therefore, we analyzed the ability of the *N*-methyl-D-aspartate (NMDA) receptor antagonists, MK-801 and ifenprodil to attenuate the TAN-67-induced efflux of accumbal dopamine.

As there is hard evidence that free radicals cause the release of excitatory amino acids (Pellegrini-Giampetro et al., 1988; Gilman et al., 1992), we finally investigated whether the free radical scavenger *N*-2-mercaptopropionyl glycine (N-2-MPG) could inhibit or prevent the TAN-67-induced efflux of accumbal dopamine. Indeed, it has recently been found that δ opioid receptor agonists such as TAN-67 and BW373U86 generate some effects via a free radical mechanism that is only partially or not at all dependent on δ opioid receptor activation (Patel et al., 2001, 2004).

All the experiments together allowed us to study the mechanisms giving rise to the TAN-67-induced increase of extracellular amount of dopamine in the NAc and to establish whether or not these mechanisms are related to (anti)nociception.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats ($n=176$; NRC Haruna, Gunma, Japan) weighing between 220 and 250 g at the start of the experiments were used. These were kept at constant room temperature (23±2 °C) and relative humidity (55±5%) under a 12-h light/dark cycle (light on at 07:00 h), with free access to food and water. The total number of rats used was 210 and only data of rats with correctly placed dialysis probes ($n=176$) were included in the analysis.

Surgery

Rats were anesthetized with sodium pentobarbitone (50 mg/kg i.p.). The anesthetized animals were placed in a stereotactic apparatus, and a guide cannula was implanted just above the NAc (AP 10.6 mm, ML 1.5 mm, DV 4.0 mm from interaural line; Paxinos and Watson, 1986) according to previously described procedures (Fusa et al., 2002; Saigusa et al., 1997, 1999, 2001). To avoid the ventricular system, cannulae directed at the NAc were angled 18° from the midsagittal plane. After completion of surgery, rats were allowed to recover for 7–10 days before experiments were carried out; guide cannulae, 0.4 mm o.d., were kept patent by stainless steel inserts. Each animal was used only once.

The experiments were performed in accordance with institutional guidelines in the care and use of experimental animals that are in compliance with the U.K. Animals (Scientific Procedures) Act, 1986, and all efforts were made to minimize animal suffering, and to reduce the number of animals used.

Dialysis and neurochemical measurements

A commercially available I-shaped removable-type dialysis probe (2 mm length cellulose membrane, 0.22 mm o.d., 50,000 mol. wt. "cutoff"; Eicom A-I-8-02 type, Kyoto, Japan) was used. The experiment was started by removing the stylet from the guide cannula and inserting the dialysis probe, of which just the dialysis tubing protruded from the tip (Fig. 1). The probe was secured to the guide cannula by a screw. Each rat was then placed in a Plexiglas box (30×30×35 cm), and inlet and outlet tubes were connected to a swivel located on a counterbalanced beam to minimize discomfort. The probe was perfused at a rate of 2.0 μl/min with modified Ringer solution (NaCl 147 mM, KCl 4 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1.1 mM; pH 7.4) and the outflow connected by Teflon tubing to a high-performance liquid chromatography system (Eicom).