Abstract  Paroxetine, a selective serotonin (5-HT) reuptake inhibitor, increased extracellular 5-HT and dopamine levels, as determined by microdialysis, in the medial prefrontal cortex (mPFC) of freely moving rats. There was a difference in the time course of the maximum response between the 5-HT and dopamine levels after paroxetine administration. The extracellular dopamine concentration reached its maximum 20 min after the peak effect of 5-HT had appeared. The paroxetine-induced increase in extracellular dopamine concentration, but not 5-HT concentration, was inhibited by the 5-HT3-receptor antagonist granisetron. These results suggest that the increase in extracellular dopamine concentration in the mPFC elicited by paroxetine is the result of stimulation of 5-HT3 receptors by the extracellular accumulation of 5-HT in the mPFC.

Keywords  Paroxetine · Serotonin reuptake inhibitor · Serotonin · Dopamine · Frontal cortex · Microdialysis

Introduction

Paroxetine, a selective serotonin reuptake inhibitor (SSRI), has been used widely in the treatment of depression (Nemeroff 1993) although it is also effective in other indications, namely panic disorder (Ballenger et al. 1998; Lecrubier et al. 1997; Oehrberg et al. 1995), obsessive compulsive disorder (Zohar and Judge 1996) and, most recently, social anxiety disorder/social phobia (Ringold 1994; Stein et al. 1996; Mancini and Ameringen 1996). The antidepressant action of SSRIs involves increased availability of 5-HT in the synapse, in part as a result of the selectivity for 5-HT transporter, whereby SSRIs have fewer side-effects than tricyclic antidepressants. However, indirect actions of SSRIs on other neurotransmitters can not be ruled out: 5-HT facilitates, for instance, the release of dopamine in the nucleus accumbens (Parsons and Justice 1993). Zazpe et al. (1994) have reported that 5-HT3 antagonists such as granisetron block the enhanced K+-evoked [3H]-dopamine release from rat olfactory tubercle slices induced by 2-methyl-5-HT, a 5-HT3 agonist, suggesting that 5-HT causes the dopamine release via the 5-HT3 receptor. Furthermore, dopamine in the nucleus accumbens and frontal cortex is thought to be involved in the central mediation of reinforcement/reward and, possibly, depression and the mechanism of action of antidepressants (Kapur and Mann 1992). In light of these observations, the present study examined the effects of paroxetine on dialysate levels of 5-HT and dopamine in the frontal cortex of freely moving rats.

Methods

Animals. Male Wistar rats (Clea Japan, Tokyo, Japan) weighing 220–300 g were used. Rats were housed in groups of five and maintained at a temperature 25±2 °C on a 12 h light-dark cycle (lights on between 7 a.m. and 7 p.m.). Rats were fed a standard laboratory diet and tap water ad libitum. The experimental procedures for animals were conducted in accordance with the guidelines of the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

Surgery. Rats were anaesthetised with pentobarbitone sodium (40 mg/kg i.p.) and placed in a stereotaxic apparatus. Rats were implanted with a guide cannula in the medial prefrontal cortex (mPFC) [A 3.2, L 0.9, V –3.0 from dura, according to the atlas of Paxinos and Watson (1986)]. The animals were allowed to recover from surgery for 7 days.

Microdialysis. Microdialysis and subsequent chromatographic analysis were performed using an automated on-line sample injection system. On the day of the experiment, rats were transferred to a plastic cage and a microdialysis probe (membrane length 2 mm; molecular mass cutoff, 50 kDa; Eicom, Kyoto, Japan) inserted into the guide cannula so that the final placement of the probe was in the mPFC. The probe was perfused at a rate of 2 µl/min with Ringer’s solution (in mM: 147 NaCl, 4.0 KCl and 2.3 CaCl2) and the dialysate collected at 20-min intervals. Dialysate samples were analysed for 5-HT and dopamine concentrations using HPLC with electrochemical detection (ECD-300) attached to a data processor (EPC-300, Eicom). A graphite working electrode (WE-3G, Eicom)
was maintained at 450 mV against an Ag/AgCl reference electrode. The mobile phase consisted of 0.1 M phosphate buffer (pH 6.0) containing 500 mg/l sodium 1-octanesulphonate and 50 mg/l EDTA-Na₂ was pumped at a rate of 2 ml/min through a reverse-phase column (Eicompak CA-50DS, Eicom). The sensitivity of the assay for 5-HT and dopamine was 50 fg/sample in each case. Drugs were administered after 2 h perfusion, i.e. when basal release was stable.

**Drugs.** Paroxetine hydrochloride and granisetron hydrochloride were obtained from SmithKline Beecham. Drugs were dissolved in sterile water and administered intraperitoneally in a volume of 1.0 ml/kg.

**Statistics.** Data are presented as the mean (±SEM) percentage of the mean amount in the three samples prior to drug injection. Experimental data were analysed using one-way analysis of variance followed by Dunnett’s or Tukey’s test. \( P<0.05 \) was considered significant.

**Results**

The basal extracellular 5-HT levels in the mPFC were 1.630±0.097 pg/sample \((n=36)\). As shown in Fig. 1, paroxetine (4 or 8 mg/kg i.p.) produced a dose-dependent increase in extracellular 5-HT levels in the mPFC. By 20 min after administration of paroxetine (8 mg/kg i.p.), extracellular 5-HT concentration was already significantly increased compared with vehicle-treated controls and increased to a maximum of 299.1±40.9\% \((n=6)\) of baseline values at 40 min post-injection. The increase persisted up to 180 min after administration of paroxetine.

Basal extracellular dopamine level in the mPFC was 0.512±0.036 pg/sample \((n=36)\). Paroxetine (4 mg/kg i.p.) increased extracellular dopamine concentration slightly but significantly from 60 to 180 min after administration. At 8 mg/kg i.p. paroxetine significantly increased extracellular dopamine concentration from 40 to 180 min after administration with a maximum of 272.1±10.3\% \((n=6)\) of baseline values being reached 60 min post-injection.

The 5-HT₃ receptor antagonist granisetron (0.5 mg/kg i.p.) did not by itself affect the extracellular 5-HT and dopamine levels in the mPFC (data not shown). Simultaneous treatment with granisetron (0.5 mg/kg i.p.), how-