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Short communication

Chronic treatment with citalopram induces noradrenaline receptor hypoactivity. A microdialysis study

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Abstract

To investigate whether chronic citalopram administration influences the cyclic AMP (cAMP) synthesis in vivo, microdialysis was used to assess citalopram-induced alterations in extracellular concentrations of cAMP in the dorsal hippocampus of freely moving rats. Citalopram administration for 4 weeks (40 mg/kg p.o. daily) did not affect the baseline levels of cAMP but decreased the noradrenaline-induced enhancement of cAMP levels. No change in forskolin-induced enhancement of cAMP levels was observed. Citalopram in situ did not exert any effect on the cAMP levels. These data support the hypothesis that chronic administration of antidepressants alters the function of noradrenergic receptors.

Keywords: 5-HT (5-hydroxytryptamine, serotonin) reuptake inhibitor, selective; β-Adrenoceptor; Dorsal hippocampus; Microdialysis

1. Introduction

A well-established neurochemical correlate of chronic treatment with a variety of antidepressants is a reduced number/function of β-adrenoceptors (Garcha et al., 1985; Mørk et al., 1990; Duncan et al., 1993; Sapena et al., 1994). This effect is controversial with respect to the selective serotonin reuptake inhibitors (Garcha et al., 1985; Goodnough and Baker, 1994; Sapena et al., 1994).

The action of selective serotonin reuptake inhibitors on presynaptic uptake of 5-hydroxytryptamine (5-HT) can be readily detected in vitro, but this effect does not correlate with the onset of the therapeutic action. Therefore, it is most likely that during long-term treatment this inhibition of 5-HT uptake leads to neurochemical adaptations involved in the mechanism of action of selective serotonin reuptake inhibitors. Although these agents preferably inhibit 5-HT uptake compared with noradrenaline and dopamine, and it is anticipated that these agents possess no or minimal effects on neurotransmitter receptors, some studies indicate that treatment with selective serotonin reuptake inhibitors affects the function of β-adrenoceptors (Koe et al., 1987; Baron et al., 1988; Byerley et al., 1988; Alhaider and Mustafa, 1991). In consistence, a functional linkage between noradrenergic and serotonergic neuronal systems has been suggested. Down-regulation of β-adrenoceptors by antidepressants has been reported to depend on intact serotonergic neurons (Brunello et al., 1982; Janowsky et al., 1982).

The previous studies led us to investigate whether citalopram could affect the ability of noradrenergic receptors to stimulate the cAMP accumulation. This was done by using a novel technique, in vivo microdialysis, to measure the levels of cAMP in the dorsal hippocampus of freely moving rats. This approach makes it possible to obtain a more physiological picture of the biochemical events occurring in the dorsal hippocampus after treatment with an antidepressant. Furthermore, forskolin was used to exclude any potential effect of citalopram on the catalytic protein of adenylate cyclase.

2. Materials and methods

2.1. Animals and drug treatment

Male Sprague-Dawley rats, initially weighing 180–200 g, were used. The control and citalopram group were

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housed under a 12-h light/dark cycle with food and tap water available ad libitum. Since citalopram was administered in the diet, the rats were observed for 4 days prior to initiating the treatment to determine their approximate food intake. Twice weekly the amount of citalopram in the diet was adjusted according to the body weight and the food intake for the last 3–4 days. The dose regimen for citalopram was 40 mg/kg daily for 4 weeks. The actual consumed amount of citalopram was calculated to be 40.1 ± 4.4 mg/kg daily.

To investigate whether citalopram in situ influenced the cAMP production the drug was introduced into the extracellular fluid by including citalopram (10 μM) in the perfusion solution.

The animal experiments met the guidelines of the governmental agency.

2.2. Microdialysis

Two days before the treatment was completed, the rats were anesthetized with Mebumal (60 mg/kg i.p.) and intracerebral guide cannulas (CMA/12) were stereotaxically implanted into the dorsal hippocampus anteroposterior −4.3 mm from bregma, lateral −2.6 mm, dorsoventral −2.2 mm (Mørk and Geisler, 1995). Anchor screws and acrylic cement were used for fixation of the guide cannulas. The body temperature of the animals was monitored by a rectal probe and maintained at 37°C. The rats were allowed to recover from surgery for 2 days, housed singly in cages.

On the day of experiment a microdialysis probe (CMA/12, 0.5 mm diameter, 2 mm length) was inserted through the guide cannula. The probes were connected via a dual channel swivel to a microinjection pump. Perfusion of the microdialysis probe with filtered Ringer solution (124 mM NaCl, 5.4 mM KCl, 0.6 mM $MgSO_4$, 25 mM $NaHCO_3$, 1 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1 mM 3-isobutyl-1-methylxanthine, pH 7.4) was initiated shortly before insertion of the probe into the brain and continued for the duration of the experiment at a constant flow of 2 μl/min. Hippocampal dialysates were collected every 15 min. After a 2-h period of stabilization, the probe was perfused with noradrenaline (1 mM) dissolved in Ringer solution for 15 min. 1.5 h after start of noradrenaline perfusion, the probe was perfused with Ringer solution containing forskolin (20 μM) for 15 min. The extracellular levels of cAMP were monitored for 5 h after the probe implantation.

After the experiments, the rats were killed by decapitation, the brains removed, and the position of the probes confirmed.

The dialysates were stored at −80°C until determination of cAMP. The aliquots were assayed for cAMP in duplicates by a commercial radioimmunoassay kit (^{125}I-cAMP) (Amersham).

2.3. Data analysis

Student's *t*-test was used for statistical evaluations of cAMP data. *P* values of less than 0.05 were considered significant.

2.4. Materials

Noradrenaline and forskolin were purchased from Sigma Chemical Co., St. Louis, USA, 3-isobutyl-1-methylxanthine from Aldrich-Chemie, Germany, and guide cannulas and microdialysis probes were from CMA/Microdialysis, Stockholm, Sweden. Citalopram was from H. Lundbeck, Copenhagen, Denmark.

3. Results

As previously reported perfusion of Ringer solutions containing noradrenaline (1 mM) or forskolin (20 μM) through the probe for 15 min produced rapid increases in the extracellular levels of cAMP in the dorsal hippocampus (Mørk and Geisler, 1995). To confirm that the peak levels of cAMP were to be found as previously reported (Mørk and Geisler, 1995), the cAMP levels were measured in all the collected fractions from three rats (results not shown).

Chronic citalopram treatment had no effect on either baseline or forskolin-stimulated cAMP levels in the dorsal hippocampus of freely moving rats (Fig. 1). However, as shown in the figure chronic citalopram treatment significantly decreased the noradrenaline-induced enhancement of the cAMP levels.

No change in the cAMP baseline and stimulated levels of cAMP was observed when the drug was introduced into the extracellular fluid in hippocampus by including citalopram (10 μM) in the perfusion solution (results not shown).

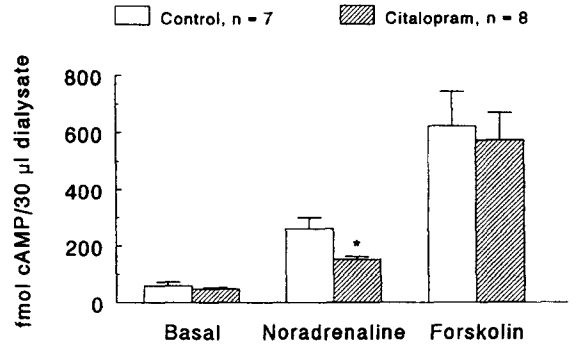

Fig. 1. Effect of chronic citalopram treatment on levels of cAMP in the dorsal hippocampus. Baseline, noradrenaline-, and forskolin-stimulated levels of cAMP in the dialysate from the dorsal hippocampus were measured in control ($n = 7$) and citalopram-treated ($n = 8$) rats. Levels of cAMP are expressed as fmol cAMP per 30 μl dialysate. * Significantly different from control. The data are mean ± S.E.M. of *n* rats.

4. Discussion

As described in the Introduction, a functional linkage between noradrenergic and serotonergic neuronal systems has been suggested (Brunello et al., 1982; Janowsky et al., 1982). According to this postulate, the therapeutic effect elicited by repeated administration of selective serotonin reuptake inhibitors may not exclusively be due to adaptations in serotonergic pathways, but may also involve changes in noradrenergic functions. Alternatively, selective serotonin reuptake inhibitors may act immediately on the noradrenergic system. Jordan et al. (1994) reported increased extracellular levels of noradrenaline in rat prefrontal cortex after i.p. administration of fluoxetine. It has, furthermore, been found that chronic treatment with fluoxetine (Byerley et al., 1988) and paroxetine (Dennis et al., 1994) decreased the number of β-adrenoceptors in rat frontal cortex. In another study, no change in the number of rat cerebral cortical β-adrenoceptors, but a decreased isoprenaline-stimulated cAMP production was observed, suggesting an impaired receptor function (Baron et al., 1988). A study from Asakura et al. (1987) indicated that extracellular 5-HT plays a role in preservation of the down-regulated state of the β-adrenoceptor induced by antidepressants from rapid reversibility. However, some studies have not been able to detect any effect of selective serotonin reuptake inhibitors on the number or function of β-adrenoceptors (Garcha et al., 1985; Goodnough and Baker, 1994; Sapena et al., 1994).

In this study we used microdialysis, which is a well-established method for measuring cAMP synthesis in brain cells of living animals (see Mørk and Geisler, 1995). Chronic treatment with citalopram was observed to decrease noradrenaline-induced cAMP accumulation in the dorsal hippocampus of freely moving rats. No effect was observed when stimulating the adenylate cyclase directly with forskolin, indicating that citalopram decreased the number or the function of β-adrenoceptors.

Stable concentrations of citalopram can be achieved by administrating the drug in the diet. Citalopram in the diet in a dose of 40 mg/kg daily has been shown to give plasma levels of citalopram close to the average steady state plasma level in patients at the standard dosis of 40 mg daily (Fredricson Overø, 1982a). It is thus possible that this dosing regimen in rats is required to cause the neurochemical adaptations assumed to occur in man. In consistence, Byerley et al. (1988) observed a concentration-dependent decrease in β-adrenoceptors after chronic treatment with fluoxetine.

Citalopram was not withdrawn 24 h prior to the experiments partly because of its short half-life in rats (3 h) (Fredricson Overø, 1982b) and partly because of the possibility that citalopram-induced adaptations could disappear within the 24 h as reported for other antidepressants (Asakura et al., 1987). This was also done to mimic the condition in the brain of patients in chronic treatment with antidepressants. The duration of treatment was set to 4 weeks to increase the possibility of observing chronic citalopram-induced adaptations.

Studies which have failed to detect any changes in the number or function of β-adrenoceptors (Garcha et al., 1985; Sapena et al., 1994) have withdrawn the selective serotonin reuptake inhibitor 24 h before the experiments. Furthermore, Sapena et al. (1994) and Garcha et al. (1985) have used low doses of citalopram (1 mg/kg daily and 10 mg/kg daily, respectively).

In addition to the above mentioned differences, we have performed the analysis in vivo. This could have allowed us to observe adaptations not readily detectable in vitro.

In conclusion, this study demonstrates that chronic citalopram treatment decreases the ability of noradrenaline to stimulate the cAMP signaling system in the brain of freely moving animals. This change may be a result of long-term administration, since citalopram in situ, administered into the hippocampus via the dialysis probe, did not exert any effect on the cAMP levels. These data support the hypothesis that chronic administration of antidepressants alters the function of β-adrenoceptors.

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