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Role of $GABA_B$ receptors in the endomorphin-1-, but not endomorphin-2-, induced dopamine efflux in the nucleus accumbens of freely moving rats

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Abstract

In vivo microdialysis was used to study the effects of the locally applied $GABA_B$ receptor antagonist 2-hydroxysaclofen and $GABA_B$ receptor agonist baclofen on the basal dopamine efflux as well as on the endomorphin-1- and endomorphin-2-induced dopamine efflux in the nucleus accumbens of freely moving rats. 2-Hydroxysaclofen (100 and 500 nmol) increased basal dopamine efflux. Baclofen (2.5 and 5 nmol) failed to affect basal dopamine efflux. 2-Hydroxysaclofen (1 and 10 nmol) which did not alter the basal dopamine efflux, enhanced the endomorphin-1 (25 nmol)-induced dopamine efflux. Baclofen (2.5 and 5 nmol) failed to affect endomorphin-1 (25 nmol)-induced dopamine efflux, but it counteracted the 2-hydroxysaclofen-induced increase of the endomorphin-1-elicited dopamine efflux. Neither 2-hydroxysaclofen (10 nmol) nor baclofen (5 nmol) affected the endomorphin-2 (25 nmol)-induced dopamine efflux. The doses mentioned are the total amount of drug over the infusion period that varied across the drugs (25 or 50 min). These results suggest that accumbal $GABA_B$ receptor plays an inhibitory role on the basal as well as the endomorphin-1-elicited accumbal dopamine efflux. The present results support our earlier reported notion that endomorphin-1 and endomorphin-2 increase accumbal dopamine efflux by different mechanisms. Finally, it is suggested that a decrease of endogenous accumbal GABA reduces the accumbal $GABA_B$ receptor-mediated GABA-ergic inhibition, enhancing thereby the accumbal dopamine efflux.

Keywords: $GABA_B$ receptor; Nucleus accumbens; Dopamine efflux; Endomorphin-1; Endomorphin-2; (Rat)

1. Introduction

The nucleus accumbens is one of the terminal areas of the mesolimbic dopaminergic neurons that originate from the ventral tegmental area. The nucleus accumbens also receives prominent non-dopaminergic, non-γ-aminobutyric acid (GABA)-ergic, excitatory afferents from limbic nuclei, such as amygdala, hippocampus and prefrontal cortex (amygdala: Robinson and Beart, 1988; hippocampus: Blaha et al., 1997; prefrontal cortex: Christie et al., 1985). The nucleus accumbens sends GABA-ergic efferents to the ventral pallidal area as well as the ventral tegmental area (Berendse et al., 1992; Zahm and Heimer, 1993). There are two types of GABA containing striatal cells, called spiny and aspiny GABA-ergic nerve cells on which GABA receptors are located (Schwarzer et al., 2001). It is suggested that the former cells are accumbal efferents (Chang and Kitai, 1985) and that the latter cells are interneurons (Bolam et al., 1983; Kita and Kitai, 1988). The nucleus accumbens contains at least two types of GABA receptor, namely $GABA_A$ and $GABA_B$ receptors (Matsumoto, 1989).

The nucleus accumbens also contains various subtypes of opioid receptor: mu-, kappa- and delta-opioid receptors. An immunohistochemical study (Svingos et al., 1997) has revealed

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that mu-opioid receptors in the nucleus accumbens of rats are localized on plasma membrane of neurons and nerve terminals of which many contain GABA. The localization of mu-opioid receptors suggests that these receptors may act as presynaptic receptors (Svingos et al., 1997).

In a previous study (Aono et al., in press), we have analyzed the role of $GABA_A$ receptors in modulating the basal dopamine efflux as well as the putative mu-opioid receptor agonist (Zadina et al., 1997) endomorphin-1- and endomorphin-2-induced dopamine efflux in the nucleus accumbens of freely moving rats. In short, we have found evidence that (1) muscimol acts at $GABA_A$ receptors on GABA-ergic interneurons that exert an inhibitory control of dopaminergic neurons and, accordingly, disinhibits these dopaminergic neurons, and that (2) bicuculline acts directly at $GABA_A$ receptors on dopaminergic neurons and, accordingly, removes the inhibitory control of these dopaminergic neurons. Furthermore, we have found evidence in favour of the hypothesis that activation of mu-opioid receptors on GABA-ergic interneurons by endomorphin-1, but not endomorphin-2, reduces the GABA-ergic input and, accordingly, disinhibits the dopaminergic neuron. In the present study, we analyzed the role of $GABA_B$ receptors in this respect.

Activation of accumbal $GABA_B$ receptors has been found to induce a suppression of the locomotor activity (Wong et al., 1991), and blockade of accumbal $GABA_B$ receptors has been found to increase the dopamine levels in the nucleus accumbens (Rahman and McBride, 2002). Because enhancement of locomotor activity can be elicited by activation of accumbal dopamine receptors (Pijnenburg and van Rossum, 1973), these findings suggest that the accumbal $GABA_B$ receptors play an inhibitory role on the accumbal dopaminergic activity. Indeed, studies on the role of $GABA_B$ receptors in modulating the cocaine-induced increase of accumbal dopamine have provided additional evidence in this respect (Ashby et al., 1999).

Activation of mu-opioid receptors in the nucleus accumbens is known to induce a large and rapid increase of accumbal dopamine efflux. Fentanyl, a mu-opioid receptor agonist, increases the naloxone-sensitive dopamine efflux in the nucleus accumbens of freely moving rats (Yoshida et al., 1999). We have recently shown that the intra-accumbal infusion of endomorphin-1 and endomorphin-2 increases accumbal dopamine efflux of rats by mechanisms that fully differ. Thus, the effects of endomorphin-1 are mediated via mu-opioid receptors in the nucleus accumbens, whereas the effects of endomorphin-2 are not: the latter effects are mediated via naloxone-insensitive receptors in the nucleus accumbens (Okutsu et al., 2006).

Electrophysiological studies have shown that activation of opioid receptors reduces the tonic GABA-ergic input of mesolimbic dopaminergic neurons and, consequently, disinhibits these neurons (Gysling and Wang, 1983; Matthews and German, 1984). Behavioural studies have revealed that stimulation of accumbal mu-opioid receptors increases food intake in rats (Ackerman et al., 2003; Will et al., 2003; Znamensky et al., 2001), requiring thereby intact dopamine D1 receptors in the nucleus accumbens (Ragnauth et al., 2000). Selective inhibition of accumbal $GABA_B$ receptors is known to increase the accumbal mu-opioid receptor-mediated feeding behaviour in rats (Znamensky et al., 2001). These data strongly suggest that accumbal $GABA_B$ receptors are involved in the increase of accumbal dopaminergic activity elicited by mu-opioid receptor stimulation. However, direct evidence in favour of this hypothesis is not available. Therefore, we analyzed the effects of intra-accumbal infusion of $GABA_B$-ergic agents on the endomorphin-1-induced accumbal dopamine efflux of freely moving rats by using *in vivo* microdialysis.

First, we studied the dose-dependent effects of intra-accumbal infusion of the $GABA_B$ receptor agonist baclofen and $GABA_B$ receptor antagonist 2-hydroxysaclofen on the basal accumbal dopamine efflux. Subsequently, we studied the specificity of these effects by combining the agonist with the antagonist. Next, we studied the effects of the $GABA_B$ receptor agonist and antagonist on the increase of accumbal dopamine efflux that was elicited by intra-accumbal infusion of endomorphin-1 or endomorphin-2.

2. Experimental procedures

2.1. Animals

Male Sprague-Dawley rats (NRC Haruna, Gunma, Japan) weighing between 200 and 220 g at the start of the experiments were used. The rats were kept at constant room temperature (23±2 °C) and relative humidity (55±5%) under a 12 h day and night cycle (light on: 0700 a.m.), with free access to food and water.

2.2. Surgery

Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). The anesthetized animals were placed in a stereotaxic apparatus, and a guide cannula was implanted just above the left nucleus accumbens [AP 10.6 mm, ML 1.5 mm, DV 4.0 mm from interaural line; Paxinos and Watson, 1998] according to previously described procedures (Aono et al., in press; Fusa et al., 2002, 2005; Okutsu et al., 2006; Saigusa et al., 1997, 1999, 2001). To avoid the ventricular system, cannulas directed at the nucleus accumbens were angled 18° from the mid-sagittal plane. After completion of surgery, rats were allowed to recover for 7 to 10 days before experiments were carried out; guide cannulas were kept patent by stainless steel inserts. Each animal was used only once.

The experiments and protocols were approved by the Animal Experimentation Committee of Nihon University School of Dentistry and were performed in accordance with institutional guidelines for the care and use of experimental animals that are in compliance with the U.K. Animals (Scientific Procedures) Act, 1986. All efforts were made to minimize animal suffering, and to reduce the number of animals used.

2.3. 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. “cut-off”, Eicom A-I-6.5-02 type, Kyoto, Japan)