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BRAIN RESEARCH

Research Report

Dopamine modulates neuronal excitability pre- and post-synaptically in the rat subfornical organ

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ABSTRACT

The aims of this study were to investigate the involvement of dopamine (DA) in drinking behaviour related to body fluid balance. All experiments were performed in rats. Water intake induced by intracerebroventricular injection of angiotensin II (ANGII) was suppressed by co-injection of DA in a dose-dependent manner. RT-PCR revealed the presence of mRNAs for all known DA receptors, D_1-D_5, in the subfornical organ (SFO), a brain region that plays a key role in regulating drinking behaviour. Extracellular recordings and whole-cell patch-clamp recordings from SFO neurons showed that DA or the D_4 selective agonist PD168077 inhibited spontaneous electrical activity. The D_4 antagonist L745870 blocked DA-induced inhibition of spontaneous electrical activity in SFO neurons. Under conditions of synaptic blockade, the inhibitory effects of DA and PD168077 still remained, but the D_2/D_3 agonist quinpirole and the D_1/D_5 agonist SKF38393 had almost no effect on electrical activity. While DA induced excitation in a small number of neurons, these excitatory responses almost disappeared following synaptic blockade. All neurons with firing rates that were suppressed by DA were excited by ANGII. In voltage clamp mode, we found that DA and quinpirole, but not SKF38393, suppressed GABAergic miniature inhibitory post-synaptic currents. These results suggest that DA inhibits neuronal activity in ANGII-sensitive SFO neurons primarily through the postsynaptic D_4 receptor subtype. This may be a cause of the suppression of ANGII-induced water intake by DA. In addition, the inhibitory DA responses in SFO neurons may be modulated by presynaptic suppression of GABAergic inhibitory inputs through D_2/D_3 receptor subtypes.

1. Introduction

Dopamine (DA) is a neurotransmitter and a bioactive amine that comprises half of the catecholamine content found in the mammalian brain. DA receptors are G-protein coupled receptors that are divided into two families, the D_1-like receptors, which consist of the D_1 and D_5 subtypes, and the D_2-like receptors, which include the D_2, D_3 and D_4 receptor subtypes.

Water ingestion related to body fluid balance is controlled through brain regions such as the circumventricular organs and the hypothalamic nuclei. Stimulation of these nuclei modulates water intake (McKinley et al., 2004). Although DAergic innervation to hypothalamic nuclei has been reported (Bouchaud and Bosler, 1986), the effect of DA in these nuclei on drinking behaviour is poorly understood. Only a single study has demonstrated that water intake induced by dehydration is suppressed by the administration of

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DA into the zona incerta in rats (Tonelli and Chiaraviglio, 1995).

Among the circumventricular organs, the subfornical organ (SFO) is a key nucleus to control water intake for body fluid balance. The SFO receives synaptic input from the zona incerta (Tanaka and Seto, 1988) and arcuate nucleus (Rosas-Arellano et al., 1996), regions of the brain that contain the soma of DAergic neurons. Fluid deprivation induces the production of DA and its metabolites in the SFO (Kariya et al., 1992). However, it is largely unknown whether DA influences the electrical activity of SFO neurons, and if it does, which subtypes of DA receptors are involved in this neural modulation. To answer these questions, we investigated the expression patterns of DA receptor subtypes in the SFO using RT-PCR and studied the effect of DA on the electrical activity of SFO neurons in slice preparations. In addition, to understand the role of DA in regulating drinking behaviour, we investigated the effect of DA on angiotensin II (ANGII)-induced water intake and the relationship between DA and ANGII in inducing responses in SFO neurons.

2. Results

2.1. *Effect of DA on water intake induced by intracerebroventricular (ICV) injection of ANGII*

The effect of DA on drinking behaviour induced by ICV injected-ANGII was tested. The total amount of water intake 1 h after the injection of saline, ANGII, DA, or DA and ANGII together was evaluated. 0.1 nmol ANGII increased water intake significantly compared to saline treatment (Fig. 1A). Co-injection of DA with ANGII suppressed the ANGII-induced water intake in a dose-dependent manner, whereas injection of 100 nmol DA by itself did not influence water intake. The volume of water intake induced by ANGII was significantly suppressed by co-injection of DA within 15 min (Fig. 1B).

2.2. *Expression of DA receptor subtypes in SFO tissue*

To investigate the expression of DA receptor subtypes in the SFO, RT-PCR was performed on RNA isolated from SFO using primers specific for the D_1–D_5 receptor subtypes. All DA receptor subtypes were found in the SFO (Fig. 2). RT-PCR of the D_2 receptor subtype revealed the presence of long and short PCR products. RT-PCR based sequence analysis showed that these bands arose from fragments of DA receptors. These data suggest that the SFO contains mRNAs that encode all of DA receptor subtypes.

2.3. *Extracellular recordings from SFO neurons*

A total of 38 multi-units from the SFO slices were analysed for dose-dependent responses to DA. Of 38 SFO multi-units, DA suppressed the neural activity of 25 (66%) multi-units in a dose-dependent manner, whereas it facilitated the neural activity of four (11%) multi-units (Figs. 3A–C). The other nine multi-units exhibited no change in their neural activity in response to DA.

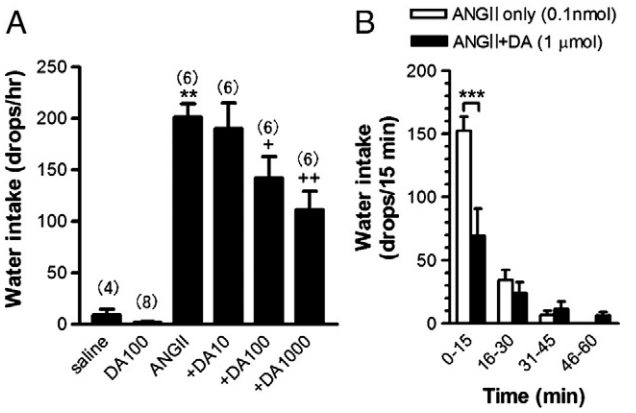

Fig. 1 – Water intake following ICV injection of DA and/or ANGII. A. ICV injection of ANGII (0.1 nmol, 4 μl) resulted in a significant increase in water intake during 1 h, compared to saline injection. The increased water intake induced by ICV-injected ANGII was significantly suppressed by 100 nmol DA and 1000 nmol DA. Note that 100 nmol DA by itself has no effect on water intake. B. Temporal changes in water intake after ICV injections of ANGII or co-injection of ANGII and DA every 15 min. Water intake following ICV-injected ANGII (0.1 nmol) was significantly suppressed by co-injection of DA (1 μmol) within the first 15 min. ** and * represent $P<0.01$ and $P<0.001$, respectively, compared to saline injection. + and ++ represent $P<0.05$ and $P<0.01$, respectively, compared to ANGII injection. Numbers in parentheses indicate the number of data. Fifteen drops are approximately equal to 1 ml.**

To investigate whether the effect of DA occurred through presynaptic or postsynaptic mechanisms, a low Ca^{2+}-high Mg^{2+}solution was used (Okuya et al., 1987). Of the 25 multi-units that showed inhibitory responses to DA in normal perfusion solution, 22 (88%) maintained their inhibitory responses in a solution containing low Ca^{2+}-high Mg^{2+}. The other three multi-units no longer responded to DA in low Ca^{2+}-high Mg^{2+} solution. In contrast, all four multi-units that showed excitatory

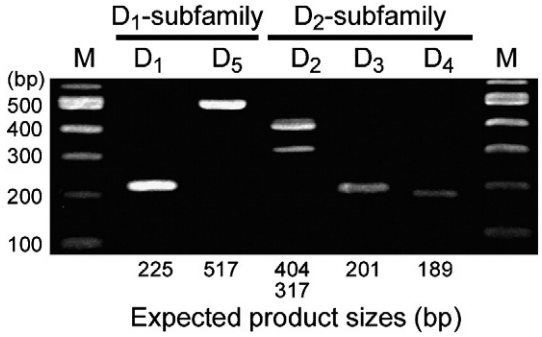

Fig. 2 – Identification of DA receptor subtypes, D_1–D_5 in rat SFO tissue. This panel shows the result of agarose gel electrophoresis of fragments amplified by RT-PCR using subtype-specific primers. PCR products representing all five receptor types were found in SFO tissue. M: molecular markers of 100 to 500 bp lengths (in 100 bp increments).