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α-Melanotropin hormone inhibits the binding of [^{3}H]SCH 23390 to the dopamine D_1 receptor in vitro

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Abstract

We have previously demonstrated that the simultaneous presence of α-melanocyte stimulating hormone (α-MSH) and dopamine resulted in a reduction in cyclic AMP (cAMP) levels in slices containing caudate putamen and accumbens nuclei as compared to those treated only with dopamine or α-MSH. This study was carried out to explore if the interaction between α-MSH and dopamine could be explained on the basis of a direct interaction between α-MSH and the dopamine D_1 receptor. Saturation curves for [*n-methyl*-^{3}H](*R*)-(+)-8 chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol hemimaleate ([^{3}H]SCH 23390) binding in the presence of increasing concentrations of α-MSH were performed. Nonlinear regression in the presence of α-MSH revealed an increased dissociation constant (K_d). The binding capacity (B_{max}) was not affected by the peptide. These data suggest an apparent competitive interaction between α-MSH and [^{3}H]SCH 23390 in striatal membranes on the dopamine D_1 receptor; ($K_i = 1.2 \times 10^{-7}$ M). The present data show that α-MSH could interact with the dopamine D_1 receptor modulating allosterically the affinity of [^{3}H]SCH 23390 for the receptor or by causing a change in the lipid environment of the dopamine receptor, resulting in an inhibition of the ligand binding to it.

Keywords: α-MSH (α-melanocyte-stimulating hormone); Striatal membranes; Dopamine D_1 receptor; [^{3}H]SCH 23390

1. Introduction

Adenocorticotophic hormone (ACTH), α-melanotropin hormone (α-MSH) and related neuropeptides mediate a diverse spectrum of biological activities in the central nervous system in mammals (Akil et al., 1980; De Wied and Jolles, 1982; Van Wimersma Greidanus et al., 1983; Torre and Celis, 1986; Gispen and Isaacson, 1993; Gonzalez et al., 1993) and these effects could be due to an interaction between melanocortins and different neurotransmitters (Torre and Celis, 1988; Lezcano et al., 1993).

It has been reported that ACTH-(1–24) inhibits the binding of ligands to the opioid receptor (Terenius et al., 1975; Gispen et al., 1976; Adan et al., 1994), the muscarinic receptor (Tonnaer et al., 1986), the $GABA_A$ receptor complex (Ito et al., 1988) and the dopamine D_2 receptor (Florijn et al., 1991, 1992). Also, it has been described that ACTH-(1–24) would inhibit activation of the adenylate cyclase coupled to D_2 receptors (Florijn et al., 1993). On the other hand, we have focused our attention on some functions of α-MSH, and have demonstrated that the presence of this natural peptide in incubated brain slices induces an increase in cAMP (Lezcano et al., 1993). Furthermore, the simultaneous presence of α-MSH and of the dopamine D_1 antagonist (*R*)-(+)-8 chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol hemimaleate (SCH 23390) (Sokoloff et al., 1993) produces a reduction in cAMP levels as compared to slices incubated with the peptide alone (Lezcano et al., 1995). These results might indicate a physiological interaction between the peptide and the dopaminergic system, probably at the receptor level. Hence, the aim of the present study was to explore whether the interaction between α-MSH and dopamine could be explained on the basis of a direct interaction between α-MSH and the dopamine D_1 receptor. Homogenates from striatal tissue were chosen accord-

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ing to neurochemical data indicating that the striatum has moderate to high melanocortin binding sites (Tatro, 1990).

2. Materials and methods

2.1. Animals

Male Wistar Rats (200–250 g) with free access to food and water were used. The colony room was maintained at 21 ± 1°C and a 14-h light cycle with the lights turned on at 6:00 a.m.

2.2. Tissue preparation

Animals were killed by decapitation and their brains removed and placed in ice. Tissue preparation was performed as in Billard et al. (1984). Briefly, striatal tissue was excised, pooled and homogenized in 10 volumes (w/v) of ice-cold 50 mM Tris–HCl buffer, at pH 7.4, using a Potter glass Teflon homogenizer. The homogenate was centrifuged at $1500 \times g$ for 15 min. Then, the supernatant was centrifuged twice at $20{,}000 \times g$ for 10 min. All the procedures were carried out at 4°C. The final pellet was resuspended in 50 mM Tris–HCl buffer, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$. The final concentration of proteins used in each determination was 3.0 mg/ml, measured according to the method of Lowry et al. (1951).

2.3. Binding assay

The specific binding of [^{3}H]SCH 23390 was measured by a filtration technique (Billard et al., 1984). Binding was carried out in the presence of the antagonist [^{3}H]SCH 23390 at different concentrations (between 0.25 to 8.0 nM). Each assay was performed by triplicate using 1.0 ml aliquots containing 3.0 mg proteins of crude synaptosomal fraction. Nonspecific binding was measured in the presence of 100 nM unlabelled SCH 23390. The peptide was dissolved in 50 mM Tris–HCl buffer, at pH 7.4 and added at different final concentrations to the samples. After incubation, for 15 min at 37°C, samples were filtered under vacuum over Whatman GF/B filter using a Brandel M-24R filtering manifold. Samples were washed three times with 4 ml of ice-cold Tris–HCl buffer (50 mM, pH 7.4) and the radioactivity was counted in an LKB-1214-Rackbeta Counter at 48% efficiency. B_{max} and K_d values were determined by computer-aided nonlinear regression analysis of the experimental data.

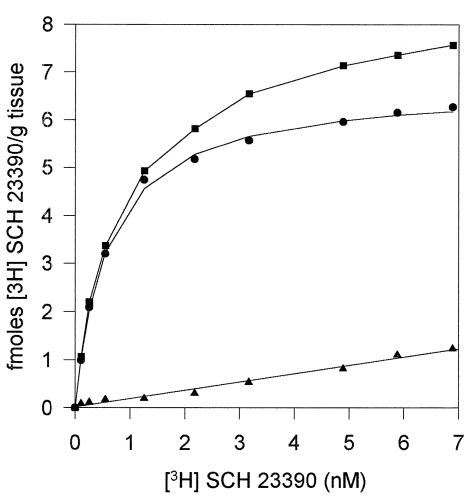

Fig. 1. Saturation of specific [^{3}H]SCH 23390 binding to dopamine D_1 receptor in rat striatum. Specific binding was defined as the difference between total binding and binding in the presence of 100 nM unlabelled SCH 23390.

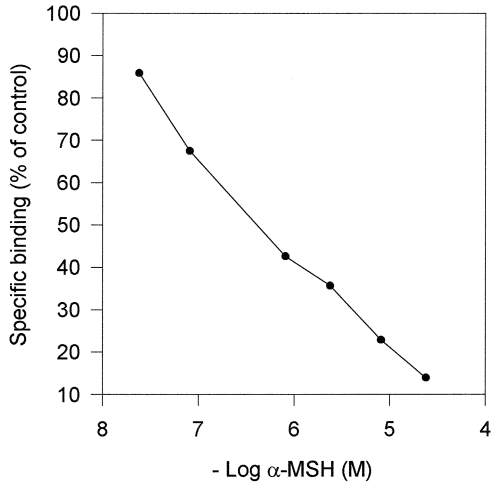

Fig. 2. Effect of increasing concentrations of α-MSH on the binding of [^{3}H]SCH 23390 to dopamine D_1 receptor in rat striatum. The data are expressed as percentage of controls added to the incubation media. Mean ± S.E.M. Each value is the mean of six observations obtained in three separate experiments.

2.4. Statistics

The results are given as percentage of controls. Statistical analysis of the data was performed by one-way analysis of variance (ANOVA), followed by Newman–Keuls test. A $P < 0.05$ was considered to indicate a significant difference.

2.5. Drugs

[*n*-Methyl-^{3}H]SCH 23390 (81.4 Ci/mmol) was obtained from New England Nuclear, unlabelled SCH 23390 was purchased from Research Biochemicals International, USA and α-MSH from Organon International (Oss, Netherlands). To avoid binding of the peptides to the glassware, only polypropylene tubes were used throughout the experiments. α-MSH and SCH 23390 were solubilized in Tris–HCl buffer, 50 mM, pH 7.4.

3. Results

3.1. Specific binding of the dopamine D_1 antagonist (SCH 23390) on rat striatal synaptosomal membranes

The specific binding of [^{3}H]SCH 23390 on rat male striatum membranes are shown in Fig. 1. Equilibrium was reached 15 min after incubation (data not shown). The binding was evaluated as a function of radioligand concentration; saturation of specific sites was clearly achieved while nonspecific binding increased linearly over the entire concentration range tested. Nonlinear regression analysis indicated the presence of a single binding site with K_d 0.59 nM and B_{max} 6.74 pmol/g tissue.

3.2. Effect of α-MSH upon binding of the antagonist [^{3}H]SCH 23390 to dopamine D_1 receptors in rat striatal synaptosomal membranes

Fig. 2 depicts the binding of the antagonist [^{3}H]SCH 23390 to striatal dopamine D_1 receptor in the presence of α-MSH in a concentration dependent manner (0.08 to 24.0 μM final concentration), with a K_i of 1.2×10^{-7} M. A nonlinear regression of the saturation curves (Fig. 3) for [^{3}H]SCH 23390 in the presence and absence of different concentrations of α-MSH (0.24, 2.4, 24.0 μM) showed an increased dissociation constant (K_d), suggesting an apparent competitive interaction between α-MSH and [^{3}H]SCH 23390 binding to the dopamine D_1 receptors. One-way ANOVA revealed a significant effect of different concentrations of α-MSH on the dopamine D_1 receptor $F_{3,11} = 23.938$, $P < 0.001$. The Newman–Keuls test showed that the K_d increased 15% ($P < 0.01$), 34% ($P < 0.01$) and 109% ($P < 0.01$) with the different concentrations of α-MSH as compared to the control (control: 0.58 ± 0.05 nM, α-MSH 0.24 μM: 0.67 ± 0.05 nM, α-MSH 2.4 μM: 0.78 ± 0.06 nM, α-MSH 24.0 μM: 1.21 ± 0.02 nM). Nonsignificant differences in the maximum density (B_{max}) between controls and the different concentrations of α-MSH were observed.

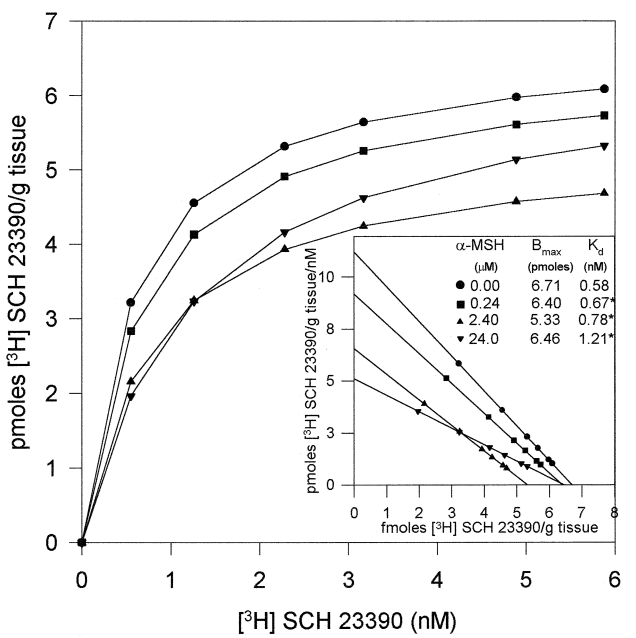

Fig. 3. Saturation curves and Scatchard plot showing the effect of various α-MSH concentrations on the affinity (K_d) of [^{3}H]SCH 23390 to the dopamine D_1 receptor in striatal membranes. The inset shows the B_{max} and K_d obtained from nonlinear regression. $* P < 0.01$ compared to control (without α-MSH) (Newman–Keuls test).

4. Discussion

Behavioral, neurochemical and neurophysiological evidence indicates that several effects of α-MSH are probably related to actions in subcortical regions, also the presence of fibers and specific binding sites in those areas support this observations (Khachaturian and Watson, 1982; Torre and Celis, 1986; Versteeg et al., 1986; Torre and Celis, 1988; Graybiel, 1990). In this connection, it is of interest that a group of five melanocortin receptor subtypes has been identified (Mountjoy et al., 1992; Gantz et al., 1993; Fathi et al., 1995). Recently, melanocortin MC_4 receptor selective analogues, as well as a ligand having a high affinity for the melanocortin MC_3 receptor, have been synthesized (Schiöth et al., 1998). Indeed, research on this area is making a significant contribution to the understanding of the mechanisms involved in the melacortin selectivity for melanocortin MC receptors.

The present data show that α-MSH inhibits the binding of [^{3}H]SCH 23390 to the dopamine D_1 receptor. ACTH-(1–24) has been found to inhibit the binding of [^{3}H]*N*-*n*-propyl norapomorphine to dopamine D_2 receptor in a concentration-dependent manner (Florijn et al., 1991). Also, the binding of the dopamine D_1 receptor antagonist [^{3}H]SCH 23390 was inhibited by ACTH-(1–24); however, this peptide was much less potent in displacing the antagonists from dopamine D_1 receptor (K_i: 1.3×10^{-5} M). Our data show that α-MSH inhibited the binding of [^{3}H]SCH 23390 to its receptor with higher potency than ACTH-(1–24) (K_i: 1.2×10^{-7} M), suggesting that dopamine D_1 receptor is particularly more sensitive to α-MSH than to ACTH-(1–24) since much higher concentrations of this peptide were needed to displace the ligand from its receptor, as compared with α-MSH. This is not surprising, insofar as ACTH acts selectively on dopamine D_2 receptors. (Sokoloff et al., 1993).

Nonlinear regression analysis showed that α-MSH inhibited [^{3}H]SCH 23390 binding to the dopamine D_1 receptor in an apparently competitive manner. It is possible that α-MSH may cause a conformational change in the receptor that masks the binding site, allosterically modulating the

affinity of [^{3}H]SCH 23390 for the dopamine D_1 receptor, by binding to a distinct site on the receptor. It is possible that part of, or some sequences of the peptide, may compete for the same binding site of [^{3}H]SCH 23390 for dopamine D_1 receptor, decreasing the affinity in the presence of α-MSH.

It has been postulated that the lipid phase plays an important role in the peptide–receptor interaction, assisting the binding of the peptide to the membrane (Verhallen et al., 1984; Sargent and Schwyzer, 1986; Gonzalez et al., 1996). In physicochemical studies, it has been described that α-MSH, a positively charged neuropeptide, interacts with interfaces containing gangliosides by penetrating monolayers formed by acidic lipids (Lezcano et al., 1996). It is possible that α-MSH interacts with the membrane lipids producing a change in the lipid environment of the dopamine D_1 receptor, which would lead to an inhibition of ligand binding to the dopamine D_1 receptor.

From the current results, we can conclude that α-MSH inhibits the binding to D_1 receptor in a dose-dependent manner and this inhibition could be related to the fact that: (a) α-MSH masks the binding sites by causing a conformational change, modulating allosterically the affinity of [^{3}H]SCH 23390 to the receptor; (b) α-MSH interacts with the membrane lipids and may cause a change in the lipid environment of the dopamine D_1 receptor, thereby resulting in an inhibition of ligand binding to this receptor; (c) a combination of both (a) and (b) is operative.

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