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Short communication

Ventral subiculum administration of the somatostatin receptor agonist MK-678 increases dopamine levels in the nucleus accumbens

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Abstract

Somatostatin (or somatotropin-release inhibitory factor, SRIF) binding and in situ hybridisation studies have indicated a high expression of receptor subtypes throughout the rat brain and, in particular, in subregions of the hippocampus and subiculum. In vitro, somatostatin and related peptides, including seglitide (MK-678), hyperpolarise subicular neurones of the burst firing type-a response, which may have functional consequences for their output. One major projection from the subiculum is to the nucleus accumbens. The functional consequence of somatostatin receptor stimulation in the ventral subiculum has been assessed by measuring extracellular levels of dopamine in the ipsilateral nucleus accumbens. In anaesthetised rats, administration of seglitide (MK-678), a somatostatin analogue with selectivity for the SRIF-1 receptor (comprising somatostatin sst2, sst3 and sst5 subtypes) significantly increased extracellular levels of dopamine in the ipsilateral nucleus accumbens shell. The result suggests that hyperpolarisation of subicular neurones by MK-678 may lead to activation of the subiculo-accumbens projection system, and an associated increase in dopaminergic function.

Keywords: Somatostatin; Subiculum; Somatostatin sst2 receptor; Dopamine; Microdialysis

1. Introduction

Somatostatin (or somatotropin-release inhibiting factor, SRIF) binding and in situ hybridisation studies have indicated a discrete regional localisation of receptor subtypes throughout the rat brain (Schindler et al., 1996). In particular, a high expression of somatostatin sst2 mRNA exist in subregions of the hippocampus and subiculum. Electrophysiological studies have shown somatostatin and related peptides, including the SRIF-1 (comprising sst2, sst3 and sst5 receptor subtypes; Hoyer et al., 1994) selective peptides seglitide (MK-678) and octreotide, hyperpolarise subiculum neurones in vitro (Greene and Mason, 1996a). Cells from this brain area show a voltage sensitive burst firing behaviour, and it was argued that hyperpolarisation of cells, which were already depolarised, may cause the membrane potential to be reduced to a level which then allows burst firing activity to be resumed, thereby causing excitation. However, it was unclear what the functional consequences would be of such an effect.

One major output of the hippocampal system, which includes the subiculum, is to the nucleus accumbens (Groenewegen et al., 1987). This projection system is glutamatergic and terminates predominantly in the shell subregion.

The aim of the present study was to determine the consequences of somatostatin receptor stimulation in the ventral subiculum on dopaminergic function in the nucleus accumbens using microdialysis. In these experiments, the effect of intrasubiculum administration of MK-678 on extracellular levels of dopamine and its metabolites was determined directly in the ipsilateral nucleus accumbens.

2. Materials and methods

Male Lister Hooded rats (Harlan, 280–300 g) were anaesthetised with urethane (1.3 g/5 ml 0.9% saline; 5

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ml/kg, i.p.) and placed in a Kopf stereotaxic frame; body temperature was maintained at 36–37°C with a heated pad and temperature controller. A microdialysis probe (cellulose acetate, active length 2 mm) and an injection cannula (28 gauge) were implanted into the left nucleus accumbens and ventral subiculum (see Fig. 1), respectively. Coordinates for surgical implantation were (from bregma and dura surface, and with bregma and lambda in the same vertical plane), nucleus accumbens: AP +1.6, LM −0.8; DV −7.2 mm; ventral subiculum: AP −6.3, LM +5.2; DV −5.0–5.5 mm.

Microdialysis probes were implanted whilst being perfused with artificial cerebrospinal fluid (aCSF), composition (in mM): NaCl (141), KCl (5), $MgCl_2$ (0.8), $CaCl_2$ (1.2), phosphate buffer (1.6), pH 7.4, and the flow-rate was 1.5 μl/min. Two hours after probe implantation, samples were collected every 15 min in vials containing 1 M glacial acetic acid (2 μl). After a further 2 h, the injection cannula, loaded with drug or vehicle, was implanted into the ventral subiculum. Drug or vehicle (aCSF) were administered in 1 μl over 2 min, 30 min later, and samples collected for a further 3 h.

At the end of the experiment Fast Green (1%) was perfused through the probe and injected through the injection cannula (0.2 μl). The animal was then decapitated and the brain removed and stored in 10% formal-saline for subsequent histological verification of placements. Data was used from animals in which probe and injection cannula placement were verified to be in the appropriate brain region.

The high performance liquid chromatography system consisted of a Rheos 4000 pump, an on-line degasser, a Higgins analytical column (75 × 2.1 mm, 3 μm C_{18}), and a Triathlon autosampler. The mobile phase consisted of a 0.15 M phosphate buffer, containing 120 mg octane sulphonic acid, 0.1 mM ethylenediaminetetraacetic acid and 5% methanol (pH 3.80). The flow-rate was 350 μl/min. Detection was accomplished with an Intro electrochemical detector (Antec) at +0.7 V, and chromatograms were integrated and stored using Millennium 2020 (Waters). Data is expressed as a percentage of a preinjection control period, obtained by averaging the last three samples prior to implantation of the injection cannula. Data was analysed by analysis of variance (ANOVA) with repeated measures. Basal levels of transmitter and metabolites were: dopamine 9.5 ± 1.2, dihydroxyphenylacetic acid (DOPAC) 8076.6 ± 707.0 and homovanillic acid (HVA) 2730.7 ± 292.0 fmol/sample.

3. Results

Application of MK-678 into the ventral subiculum produced a significant and dose-dependent increase in extracellular levels of dopamine in the ipsilateral nucleus accumbens (Main Effect Treatment: $F[df\ 2,13] = 29.17$, $P < 0.0001$). The response reached 141 ± 9% and 206 ± 22% of basal levels within 60 min following administration of 100 and 500 ng, respectively (Fig. 2A), and continued to rise, reaching about 250% of basal levels 2 h after administration of the highest dose. For the overall response profile (3 h post administration): MK-678 100 ng vs. vehicle, $F[df\ 1,13] = 11.2$, $P = 0.0053$; MK-678 500 ng vs. vehicle, $F[df\ 1,13] = 56.40$, $P < 0.0001$. The increase in dopamine release was also accompanied by a significant dose-dependent increase in extracellular levels of DOPAC (Fig. 2B; Treatment: $F[df\ 2,13] = 8.84$, $P < 0.0038$),

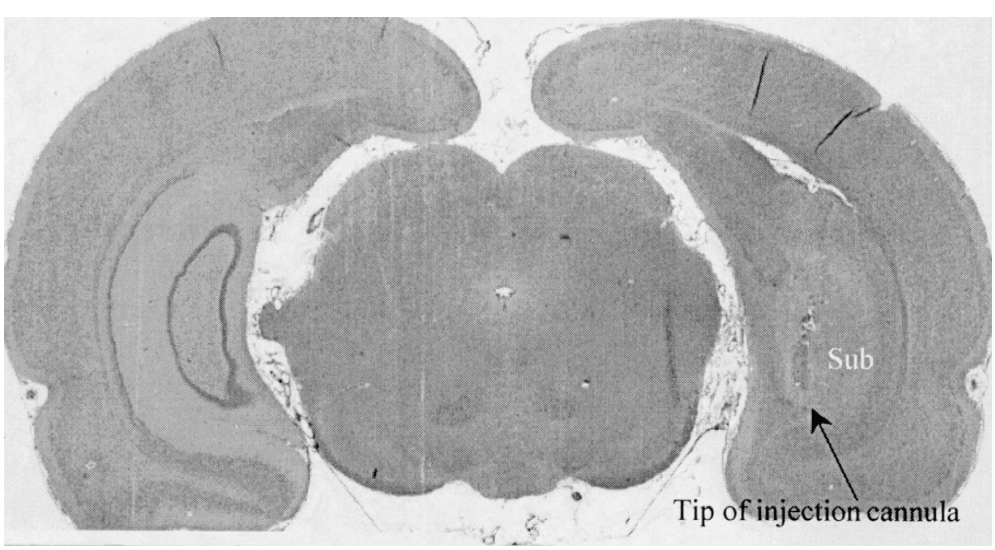

Fig. 1. Cresyl violet-stained section of rat brain (35 μm) showing placement of injection cannula in the ventral subiculum (Sub).

reaching 121 ± 3% 2 h after application of the highest dose (vs. vehicle: $F[df\ 1,13] = 14.02,\ P < 0.0025$). Extracellular levels of HVA were not significantly increased (Fig. 2C).

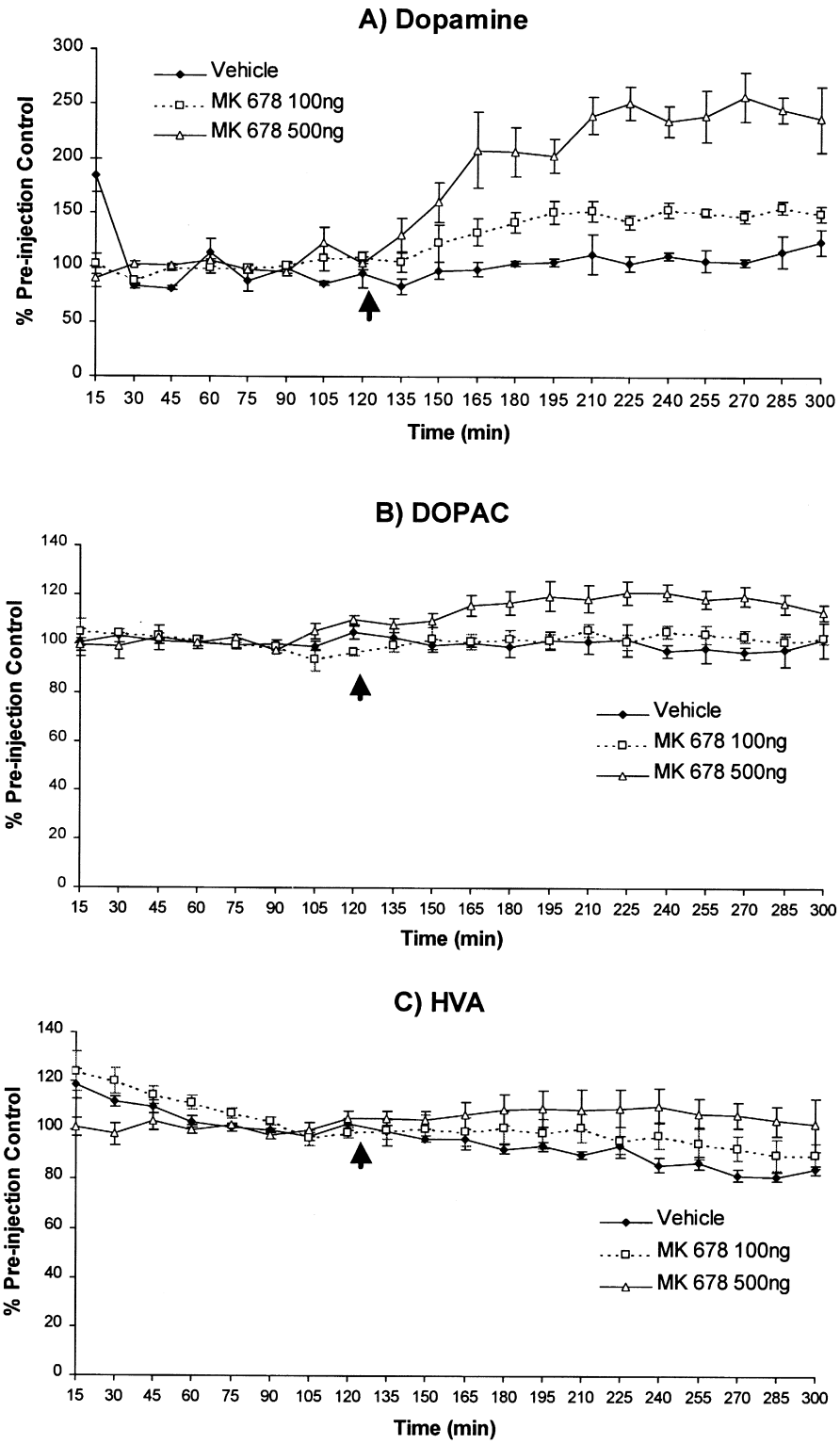

Fig. 2. The effect of MK-678 infused into the ventral subiculum on extracellular levels of: (A) Dopamine, (B) DOPAC and (C) HVA in the ipsilateral nucleus accumbens. Data expressed as a percentage of a preinjection control (see Section 2) and represents the mean ± S.E.M. (vehicle, $n = 4$; MK-678 100 ng, $n = 7$; MK-678 500 ng, $n = 5$). MK-678 significantly increased extracellular levels of dopamine at both doses, and increased the metabolite DOPAC at the highest dose (ANOVA, $P < 0.05$).

4. Discussion

Ventral subiculum administration of MK-678 evoked a pronounced and long-lasting increase in extracellular levels of dopamine in the ipsilateral nucleus accumbens. MK-678 is a short synthetic (hexapeptide) analogue of somatostatin, and is metabolically more stable; it possesses high affinity for the SRIF-1 class of receptor (comprising sst2, sst3 and sst5), but shows greater affinity for the sst2 receptor subtype (pK_i: sst2, 9.74; sst3, 7.92; sst5, 8.26; see review Hoyer et al., 1994).

Electrophysiological studies have shown somatostatin and related peptides, like MK-678 and octreotide (also SRIF-1 selective; Hoyer et al., 1994), to hyperpolarise subiculum neurones in-vitro (Greene and Mason, 1996a). The peptides appeared to have a preferential effect on cells that were characterised as being intrinsically burst (IB) firing (IB cells), and to reduce $GABA_B$ receptor-mediated synaptic potentials (Greene and Mason, 1996b). In the ventral subiculum, IB cells revert to regular spiking mode when they are depolarised close to their action potential. It was argued that hyperpolarisation of depolarised IB cells could restore burst firing, and thereby exert an excitatory effect on the pathway as a whole. Moreover, the rank order of potency for a series of peptides, suggested the response was mediated by the sst2 subtype.

The subiculum gives rise to many subcortical and cortical projections, one of which is to the nucleus accumbens (Groenewegen, et al., 1987). We provide evidence that a functional consequence of somatostatin receptor stimulation with MK-678 in the ventral subiculum is to increase dopamine release and metabolism in the ipsilateral nucleus accumbens, possibly by excitation of the projection neurones.

The hippocampal-nucleus accumbens pathway influences psychomotor activity in rats (Mogenson and Nielsen, 1984; Yang and Mogenson, 1987) and in humans, the ventral hippocampus, including the septo-hippocampal and subiculo-accumbens projection have been implicated in the aetiology of schizophrenia (Gray et al., 1991). The present work indicates that somatostatin receptor stimulation (possibly sst2) increases dopaminergic function. However, it is presently unclear whether such an increase in dopamine release will have behavioural consequences, e.g. by increasing locomotor activity, as shown previously after ventral hippocampal administration of *N*-methyl-D-aspartate (Yang and Mogenson, 1987) or carbachol (Mogenson and Nielsen, 1984), or in animal models relevant to schizophrenia, e.g. latent inhibition (Gray et al., 1995), or prepulse inhibition (Klarner et al., 1998).

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