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The anxiolytic effects of somatostatin following intra-septal and intra-amygdalar microinfusions are reversed by the selective sst2 antagonist PRL2903

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ABSTRACT

Somatostatin (SST) is a polypeptide with two biological isoforms (SST14, and SST28), and five SST receptor subtypes (sst1-5). Together, they mediate a number of neural and hormonal functions. Recently, we found that intracerebroventricular (ICV), intra-amygdalar, and intra-septal microinfusions of SST14, SST28, and a selective sst2 receptor agonist L-779976 all produced anxiolytic-like effects in the elevated plus-maze, a widely used animal model of anxiety. The receptor specificity of these anxiolytic-like effects, however, has not been conclusively established.

Accordingly, the anxiolytic effects of SST in the elevated plus-maze were assessed following intra-septal or intra-amygalar microinfusions of 1) SST (1.5 μg per hemisphere), 2) the highly selective sst2 receptor antagonist PRL2903 (1.5 μg per hemisphere), or 3) the combination of SST and PRL2903 (each 1.5 μg per hemisphere). Antagonism of the anxiolytic effects of SST in the plus-maze by PRL2903 should result in open-arm exploration that is equivalent to that of 4) vehicle-injected control rats.

Both intra-septal and intra-amygdalar microinfusions of SST produced anxiolytic effects in the elevated plus-maze, consistent with results found previously after ICV microinfusions (see Engin et al., 2008; Engin and Treit, 2009; Yeung et al., 2011). More importantly, infusion of PRL2903 completely reversed the anxiolytic effects of SST in both the amygdala and the septum. These results show that somatostatin's anxiolytic effects are mediated by sst2 receptors contained in the amygdala and septum of the rat brain.

1. Introduction

Somatostatin (SST) is a cyclic polypeptide that inhibits an array of endocrine, exocrine, and gastrointestinal functions (e.g. inhibition of growth hormone, insulin, glucagon, secretin, gastrin, and cholecystokinin, Brazeau et al., 1972; Cervia and Bagnoli, 2007; Moller et al., 2003; Selmer et al., 2000). In addition, there is now compelling evidence of SST acting as both a neuromodulator and neurotransmitter in the brain (e.g., Wang et al., 1989; Karschin 1995; Kreienkamp et al. 1997; Yeung et al., 2011). These central effects of SST are thought to be mediated by two somatostatin isoforms [i.e., SST14 and SST28] acting on five, G-protein-coupled somatostatin receptors [i.e., sst1-5], all of which are found throughout the brain (Epelbaum, 1986; Moller et al., 2003; Weckbecker et al., 2003).

SST was recently implicated in affective responses such as anxiety. Engin et al. (2008) found that intracerebroventricular (ICV) microinfusions of somatostatin produced anxiolytic effects in both behavioral and neurophysiological models of anxiolytic drug action in rats. Anxiety-like behavior in the rat elevated plus-maze was also inhibited by ICV infusions of a selective agonist of the sst2 receptor subtype, L-779976, whereas infusions of sst1, and sst3-5 agonists were without effect (Engin and Treit, 2009). Finally, the two isoforms of somatostatin found in the brain (SST14 and SST28) each produced anxiolytic-like effects in the elevated plus-maze after intracerebral microinfusions into the amygdala and septum (Yeung et al., 2011). The absence of this anxiolytic effect after identical infusions into the striatum–which also contains SST receptors–suggested some degree of site-specificity for the anxiolytic effects of intracerebral SST14 and SST28 (Yeung et al., 2011).

It seems likely that the anxiolytic-like effects of SST are mediated by sst2 receptor subtype, for three reasons. First, ICV infusion of the selective sst2 receptor agonist L-779976 produced anxiolytic effects in the plus-maze that were comparable to both SST and diazepam, a potent anxiolytic compound (Engin and Treit, 2009). Second, the sst2 receptor subtype is densely expressed in structures already implicated in anxiety, such as the amygdala and septum (Holloway et al., 1996; Selmer et al., 2000; Treit and Menard, 2000). Third, microinfusion of SST into either of these brain areas produced robust anxiolytic effects in the elevated plus-maze (Yeung et al., 2011). Despite this evidence, however, the receptor specificity of the anxiolytic

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effects of SST in general, and in the amygdala and septum specifically, has not been clearly established.

An obvious approach to establishing the receptor specificity of the anxiolytic effects of somatostatin is to determine whether such effects can be blocked by co-infusion of an sst2 antagonist. Although early attempts to establish an antagonist with selective affinity for the sst2 subtype were not particularly successful, systematic work by Coy and others eventually led to such a development. PRL2903 is a receptor antagonist with a high, *selective* affinity for the sst2 receptor subtype (e.g., Hocart et al., 1998; 1999; Rossowski et al., 1994; 1998). Functional studies have shown, for example, that PRL2903 completely blocks intracellular Ca2 + influx produced by the selective sst2 agonist L-779976, with no intrinsic effect by itself (Cheng et al., 2002). Thus, PRL2903 seemed to be an ideal pharmacological tool for determining the receptor subtype mediating the anxiolytic-like effects of SST.

The present experiments were designed to assess the role of the sst2 receptor in the anxiolytic effects of SST. Classical agonist–antagonist interaction studies were conducted in both the amygdala and septum, using SST as agonist and PRL2903 as antagonist. Complete reversal of the anxiolytic effects of SST would indicate a predominant role of the sst2 receptor in anxiety-reduction. Partial or incomplete antagonism of SST-induced anxiolysis would indicate that other receptors in addition to sst2 may be involved in the anxiolytic effects of SST. The complete absence of anxiolytic antagonism by PRL2903 would indicate–but not prove–that the sst2 subtype is not involved in anxiety reduction.

2. Materials and methods

2.1. Subjects

Subjects were 99 males, Sprague–Dawley rats, weighing 200–300 g at the time of surgery. Rats were individually housed in 47 × 25 × 20.5 cm polycarbonate cages for the duration of the experiment and maintained on a 12:12 h light/dark cycle (lights on at 0600 h). Food and water were available ad libitum. The treatment of all animals was in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals, and the Canadian Council on Animal Care. All procedures were also approved locally by the Biosciences Animal Policy and Welfare Committee of the University of Alberta. Power analyses were carried out before the experiments to minimize the number of animals used, and all possible measures to minimize suffering and stress were taken during the experiments. Just prior to surgery, the rats were randomly assigned to surgery conditions (bilateral amygdalar cannulation or bilateral septum cannulation).

2.2. Surgery

Rats were anesthetized with isoflurane (5% induction, 1.5% maintenance in N2O and O2, 67% N2O and 33% O2; Halocarbon Product Corp. River Edge NJ, USA), and injected with Marcaine (1.5 mg/0.3 mL s.c.; Hospira, Quebec, Canada), and Rimadyl© (2.5 mg/0.5 mL s.c: Pfizer, Quebec, Canada), just under the cranial cutaneous membranes, and placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). Following hydration with 0.9% saline (4 cm^3, i.p.), an incision was made to expose skull. The subjects were then bilaterally implanted with Stainless-steel 22-gauge guide cannulae (Plastics One, Roanoke, VA, USA) targeting the amygdala (AP: −2.5, ML: −4.2, DV: −6.6), or the septum (AP: 0.7 mm, ML: −2.6, DV: −4.2, angled 22° towards the midline), using Paxinos and Watson's (1986) stereotaxic atlas of the rat brain. The cannulae were lowered to within 0.5 mm of their intended targets and secured to the skull with three jeweler's screws and cranioplastic cement. A dummy cannula was inserted into each guide cannula in order to keep the cannula tract clear. The surgical area was treated with Hibitaine® (Pfizer, Quebec, Canada), upon completion of the procedure. Following surgery, the rats were placed in a warm environment until they regained consciousness. Rats were then allowed to recover for at least 4 days in their home cages, during which time they were briefly handled to assess their general health and to check for any obstruction in the cannulae.

2.3. Infusion procedure

Just prior to behavioral testing, rats from the amygdalar and septal surgical groups were randomly assigned to one of four drug conditions: 1.) Somatostatin (SST) (AnaSpec, CA, USA), 3 μg/μL (1.5 μg per hemisphere), 2.) a selective sst2 antagonist PRL2903 (kindly donated by Dr. David Coy, Tulane Health Sciences Center, LA), 3 μg/μL (1.5 μg per hemisphere), 3.) the co-infusion of SST and PRL2903 at the same doses and concentrations as above, or 4.) a 5% DMSO vehicle solution (1 μL per hemisphere). Both SST and PRL2903 were dissolved in a 5% DMSO vehicle at final concentrations of 1 μg/μL. The drugs were infused bilaterally (1.5 μg/hemisphere) via an infusion pump (Harvard Apparatus 22, MA, USA) at a rate of 1 μL/min for 30 s per hemisphere through 26-gauge stainless-steel internal cannulae attached to a 10-μL Hamilton syringe by polyethylene tubing. The internal infusion cannulae extended 0.5 mm below the ventral tip of the guide cannulae. Drug flow was confirmed by displacement of a bubble inside the polyethylene tubing. The internal infusion cannulae were left in place for 30 s after the end of the infusion period to allow for diffusion.

2.4. Behavioral testing

The behavioral procedures were the same as those described previously (e.g., Treit et al. 1993) and below. The experimenter handled each of the rats for 5 min, checking the cannulae tracts for blockage and habituating the rats to the infusion procedures, on each of the 4 consecutive days prior testing. All behavioral testing occurred in a quiet testing room between 0900 h and 1800 h and was recorded on videotape. Testing started 10 min after the end of the infusion procedure. The experimenter always sat in a chair positioned in the same place in the room during plus-maze testing. To ensure blind behavioral coding, only the animal number and not the group it belonged to was shown in the videotape.

2.4.1. Elevated plus-maze

The maze was a plus-shaped apparatus with an open roof, consisting of two 50 × 10 cm open-arms, and two 50 × 10 × 50 cm enclosed arms, and elevated at a height of 50 cm. All testing was conducted between 0900 and 1800 h in a quiet and dimly illuminated room. Each animal was tested for 5 min. Four variables were measured: (1) time spent in the open-arms; (2) time spent in the closed arms; (3) number of entries into the open-arms; and (4) number of entries into the closed arms. A rat was considered to have entered or spent time in an arm only when all four paws were in the respective arm. The time spent in the open-arms and the number of open-arm entries were expressed as a percentage of total arm activity (open-arm time/open-arm time + closed-arm time) × 100, and total arm entries (open-arm entries/open-arm entries + closed-arm entries) × 100, respectively. Increases in the percentage of open-arm time or open-arm entries are taken as measures of anxiety-reduction (anxiolysis). In addition, the total of all arms entered, as well as the total of closed arms entered were used as indexes of general activity (Hogg, 1996; Pellow and File, 1986).

2.5. Histology

Following behavioral testing, rats were euthanized in a gas chamber with 100% N2O and perfused intracardially with 0.9% (wt/vol) saline followed by 10% (vol/vol) formaldehyde. Post-fixation, the brains were removed from the skull and placed in a 10% formaldehyde solution for at least 48 h. The brains were then frozen with dry ice and cut