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Effect of somatostatin and its antagonist on morphine analgesia in mice

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KEY WORDS *morphine; analgesia; somatostatin*

ABSTRACT

AIM: To study the effects of somatostatin (SST) and its antagonist cyclo-(7-aminoheptanoyl-Phe-*D*-Trp-Lys-Thr [Bzl]) (SSA) on morphine-induced analgesia. **METHODS**: The pain assays were the hot plate and the tail flick test. **RESULTS**: SST or SSA *per se* administered intracerebrally at the doses of 0.1 and 1 mg/mouse did not change the pain threshold of mice both in the hot plate and in the tail flick test. However, at the higher dose (10 mg/mouse), SST and SSA decreased the pain threshold in the tail flick test only. SST and SSA administered at the dose of 0.1 mg/mouse did not change morphine-induced analgesia. By contrast, SST and SSA at the doses of 1 and 10 mg/mouse reduced morphine analgesia effects both in the hot plate as well as in the tail flick test. **CONCLUSION**: Our results indicate that SSA as well as SST may be useful in studying pain mechanisms.

INTRODUCTION

Many reports have indicated that somatostatin (SST) may play a role in pain regulation. SST has been found in the dorsal root ganglia and in the primary sensory neurons[1,2]. Iontophoretic application of SST caused a depressant action of the dorsal horn neurons[3]. Furthermore, behavioral studies have indicated that SST is able to modify the pain threshold[4,5]. Some years ago it was published a study[6] on the synthesis of cyclo-(7-aminoheptanoyl-Phe-*D*-Trp-Lys-Thr [Bzl]) (SSA), which is a peptide that displays some somatostatin antagonistic properties, *ie*, blocking the inhibitory effects of exogenous somatostatin on the release of the growth hormone, insulin, and glucagon. The availability of a somatostatin antagonist exerting opposite or different effects with respect to the SST effects in the pain study, may help to explain the role of SST in pain modulation. Therefore, we performed the present study to investigate the effects of SST and SSA on pain and on morphine-induced analgesia in mice since few data have been reported on these effects and also as part of our study of the pharmacological interferences with morphine-induced analgesia[7,8].

MATERIALS AND METHODS

Animals Male CD-1 mice (Charles River, Italy) weighing 25 – 30 g were used in the experiments. Animal care and use followed the directions of the Council of the European Communities (1986). They were maintained in a climate light controlled room [(22 ± 1) ℃, 12/12 h dark/light cycle with lights on at 7:00] and with free access to food and water prior to the experiments. Testing took place during the light phase. The animals were brought to the test room for at least 3 h before testing. Each animal was used only in one experimental session.

Pain assays The pain assays were the hot plate (HP) and the tail flick test (TF). The HP test was performed as previously described[8]. Briefly, the hot plate (Socrel Mod. DS37, Ugo Basile, Italy, 25 cm × 25 cm) was set at a plate temperature of (55 ± 0.5) ℃ to give a latency of 15 – 17 s in control animals. The time of hind paw licking was recorded, and measuring was terminated if the licking exceeded the cut-off time (60 s). The tail flick latency[9] was obtained using a tail flick unit (Socrel Mod DS-20, Ugo Basile, Italy).

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The animals were gently immobilized by using a glove, and the radiant heat was focused on a blackened spot 1 – 2 cm from the tip of the tail. Beam intensity was adjusted to give a tail flick latency of 2 – 3 s in control animals. Measuring was terminated if the latency exceeded the cut-off time (10 s) to avoid tissue damage.

Experimental procedure In all the experiments mice were tested twice 60 and 30 min before drug administration in the baseline latency determination and afterwards, 15, 30, 60, and 90 min after drug administration. SST and SSA (Sigma Chemicals CO, USA) were administered intracerebroventricularly (icv) at the doses of 0.1, 1, 10 mg/mouse alone or immediately before morphine administration. Morphine hydrochloride (Carlo Erba, Italy) was administered intraperitoneally (ip) at the dose of 5 mg/kg. The peptide purity was not checked and the concentrations were calculated according to the purity quoted from source.

On the day of the testing, all drugs used in the experimental sessions were dissolved in 0.9 % NaCl solution for ip or distilled water for icv administration. The drugs were injected in a volume of 2.5 mL/mouse for icv administration which was performed according to the method of Haley and McCormick (1957)[10]. At the end of the experimental session, the injection site was verified by using 1 % methylene blue and the distribution of the dye in the cerebrum was examined.

Statistical analysis Data obtained in the experiments were analyzed by using the analyses of variance followed by the Mann-Whitney U-test for between-group differences[9]. All the data were expressed as mean ± SEM and significance was assumed at a 5 % level.

RESULTS

In the hot plate (HP) test as well as in tail flick (TF) test, distilled water injected icv did not change the pain threshold [HP: $F_{(5,54)} = 1.24$, ns; TF: $F_{(5,84)} = 1.22$, ns]. In the HP test both SST and SSA did not change the pain threshold whereas a reduction in the response time was obtained when SST or SSA were administered at the dose of 10 mg/mouse in the TF test [SST: $F_{(5,84)} = 7.49$, $P < 0.01$; SSA: $F_{(5,84)} = 6.37$, $P < 0.01$). A subsequent analysis revealed that SST induced a significant decrease in the nociceptive threshold 15 and 30 min after administration (15 min: $U_{(15)} = 37$, $P < 0.01$; 30 min: $U_{(15)} = 52$, $P < 0.05$), whereas SSA decreased the pain threshold from 15 min to 60 min after administration (15 min: $U_{(15)} = 48$, $P < 0.01$; 30 min: $U_{(15)} = 40.5$, $P < 0.01$; 60 min: $U_{(15)} = 61$, $P < 0.05$).

Tab 1 and 2 showed the results obtained with SST or SSA in animals pretreated with morphine.

In the HP test (Tab 1), higher dose (10 mg/mouse, icv) of SST or SSA induced a significant reduction of morphine analgesia. Even though SSA reduced morphine analgesia when administered at the dose of 1 mg/mouse, this reduction is significant only

Tab 1. Effects induced by morphine (Mor, 5 mg/kg, ip), somatostatin (SST, 0.1, 1, 10 mg/mouse, icv) plus morphine, and cyclo-(7-aminoheptanoyl-Phe-*D*-Trp-Lys-ThR [Bzl]) (SSA, 0.1, 1, 10 mg/mouse, icv) plus morphine, 15, 30, 60, 90 min after drug administration in the hot plate test. In the predrug the latencies were obtained 30 min before drug administration. Results are expressed as mean ± SEM. $^bP < 0.05$; $^cP < 0.01$ *vs* Mor.

	Mor	Mor + SST			Mor + SSA		
		0.1	1	10	0.1	1	10
Predrug Time/s	15 ± 0.9	16.5 ± 1.3	14.7 ± 1.7	17.1 ± 1.3	14.2 ± 1.3	15.1 ± 1.8	15.7 ± 1.4
15	42.3 ± 2.1	37.5 ± 1.9	44.2 ± 2.1	22.3 ± 1.7^c	46.3 ± 5.8	15.1 ± 1.8	15.7 ± 1.4^b
30	38.7 ± 2.5	46.1 ± 2.5	39.5 ± 3.2	21.7 ± 1.9^b	44.2 ± 3.5	36.4 ± 3.4	27.4 ± 2.3^b
60	47.8 ± 4.1	45.7 ± 3.7	49.5 ± 4.1	23.8 ± 1.7^b	46.5 ± 3.1	33.1 ± 2.7	21.5 ± 1.9^c
90	33.5 ± 2.4	38.5 ± 2.1	36.8 ± 2.1	21.5 ± 1.7^b	32.7 ± 2.1	19.4 ± 1.3^c	23.8 ± 1.7

90 min after treatment.

In the TF test (Tab 2), morphine analgesia effects were reduced by SST or SSA administered at the doses of 1 and 10 mg/mouse from 15 min to 60 min after administration. In particular, SST administered at the dose of 1 mg/mouse antagonized morphine analgesia 30 and 60 min after treatment, whereas SST administered at the dose of 10 mg/mouse reduced morphine analgesia 15, 30, and 60 min after treatment. SSA administered at the dose of 1 mg/mouse antagonized morphine effects 15 and 30 min after the treatment whereas SSA administered at the higher dose (10 mg/mouse) reduced morphine analgesia 15, 30, and 60 min after treatment.

DISCUSSION

The results of this study indicate that SST and SSA induce the same effects on pain when administered at relatively high doses in animals. Indeed, at low doses SST and SSA did not change the pain threshold whereas at higher doses, SST and SSA reduced the response to pain stimuli. Furthermore, when administered at the same doses (1 and 10 mg), SST and SSA reduced morphine-induced analgesia.

A previous study indicated that SST administered intrathecally at a dose of 10 mg or more caused analgesia in the TF test, and this effect was associated with hind limb paralysis and neuronal damage of the spinal cord[5]. Recently, Ohkubo *et al* (1990)[11] reported that SSA administered intrathecally at a higher dose was able to reduce the licking response in the late phase of the formalin test in mice. Therefore, these data and our data confirm and extend previous studies indicating that SSA may exert somatostatin-like effects when administered at high doses in animals[12].

Our data obtained with SST in morphine pretreated animals are in agreement with those reported by Kasson and George (1983)[13] showing that SST administered icv at a dose of 40 mg/rat decreases morphine-induced analgesia. These authors also suggested that SST might interfere with the opioid system in the brain by exerting agonistic effects at low doses and antagonistic effects at higher doses on more potent opioid agonists as well as on morphine (Kasson and George, 1983)[13]. Terenius)1976)[14] indicated the possibility that SST might bind to opiate receptors with the characteristics of a partial agonist, but to our knowledge no data are available on the possible interference exerted on the opioid receptors by somatostatin antagonist SSA. The possibility that SSA also may interfere with the opioid receptors may arise from our study.

Finally, the present study provides evidence that when SST and SSA are administered at relatively high doses in animals, they induced the same effects on antinociception and on morphine-induced analgesia. This also indicates the possibilty that SSA as well as SST may be useful in studying nociception mechanisms.

Tab 2. Effects induced by morphine (Mor, 5 mg/kg, ip), somatostatin (SST 0.1, 1, 10 mg/mouse, icv) plus morphine, and cyclo-(7-aminoheptanoyl-Phe-*D*-Trp-Lys-ThR [Bzl]) (SSA 0.1, 1, 10 mg/mouse, icv) plus morphine, 15, 30, 60, 90 min after drug administration in the tail flick test. In the predrug the latencies were obtained 30 min before drug administration. Results are expressed as mean ± SEM. $^bP<0.05$; $^cP<0.01$ *vs* Mor.

	Mor	Mor + SST			Mor + SSA		
		0.1	1	10	0.1	1	10
Predrug	2.7±0.2	2.3±0.5	2.4±0.7	2.6±0.5	2.8±0.8	2.4±0.3	2.5±0.4
Time/s							
15	5.3±0.4	4.8±0.7	5.8±0.6	3.2±0.6^b	4.8±0.6	3.5±0.2^b	3.2±0.7^b
30	5.8±0.7	5.7±0.6	3.0±0.4^c	3.2±1.9^b	5.7±0.5	3.8±0.3^b	3.1±0.2^b
60	6.3±0.6	5.8±0.7	3.8±0.4^b	3.5±0.4^b	5.6±0.5	4.8±0.6	3.2±0.5^c
90	3.5±0.3	3.3±0.4	36.8±0.3	2.8±0.7	3.8±0.5	3.9±0.8	2.9±0.7

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REFERENCES

1 Hokfelt T, Elde R, Johansson O, Luft R, Nilsson G, Arimura A. Immunohistochemical evidence for separate populations of somatostatin-containing and substance P-containing primary afferent neurons in the rat. Neuroscience 1976; 1: 131 – 6.
2 Seybold VS, Elde R. Immunohistochemical studies of peptidergic neurons in the dorsal horn of the spinal cord. J Histochem Cytochem 1980; 28: 367 – 70.
3 Randic M, Miletic V. Depressant actions of methionine-enkephalin and somatostatin in cat dorsal horn neurones activated by noxious stimuli. Brain Res 1978; 152: 196 – 202.
4 Chrubasik J, Meynadier J, Blond S, Scherpereel P, Aclerman E, Weinstock M, *et al*. Somatostatin, a potent analgesic. Lancet 1984; 11: 1208 – 9.
5 Mollenholt P, Post C, Rawal N, Freedman J, Hokfelt T, Paulsson L. Antinociceptive and neurotoxic actions of somatostatin in rat spinal cord after intrathecal administration. Pain 1988; 32: 95 – 105.
6 Fries JL, Murphy WA, Sueiras-Diaz J, Coy DH. Somatostatin antagonist analog increase GH, insulin and glucagon release in the rat. Peptides 1982; 3: 811 – 4.
7 Capasso A, Di Giannuario A, Loizzo A, Pieretti S, Sorrentino L. Central interaction of dexamethasone and RU38486 on morphine antinociception in mice. Life Sci 1992; 51: PL139 – 43.
8 Pieretti S, Capasso A, Di Giannuario A, Loizzo A, Sorrentino L. The interaction of peripherally and centrally administered dexamethasone and RU-38486 on morphine analgesia in mice. Gen Pharmacol 1991; 22: 929 – 33.
9 Pieretti S, d'Amore A, Loizzo A. Long-term changes induced by developmental handling on pain threshold: effects of morphine and naloxone. Behav Neurosci 1991; 105: 215 – 8.
10 Haley TJ, McCormick WG. Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. Br J Pharmacol 1957; 12: 12 – 5.
11 Ohkubo T, Shibata M, Takahashi H, Inoki R. Roles of substance P and somatostatin on trasmission of nociceptive information induced by formalin in spinal cord. J Pharmacol Exp Ther 1990; 252: 1261 – 8.
12 Spencer GSG, Hallett KG. Somatostatin antagonist analogue stimulates growth in rats. Life Sci 1985; 37: 27 – 30.
13 Kasson BG, George R. Endocrine influences on the actions of morphine. Ⅲ. Responses to hypothalamic hormones. Neuroendocrinology 1983; 37: 416 – 20.
14 Terenius L. Somatostatin and ACTH are peptides with partial antagonist-like selectivity for opiate receptors. Eur J Pharmacol 1976; 38: 211 – 3.

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生长抑素及其拮抗剂对小鼠吗啡镇痛的影响

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