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Opiate antagonistic properties of an octapeptide somatostatin analog

(electrophysiology/hippocampus/analgesia/naloxone/binding)

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ABSTRACT **The somatostatin analog D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-NH-CH(CH_2OH)$CHOHCH_3$ (SMS 201-995) displaces [^{3}H]naloxone from its binding sites (IC_{50}, 38 ± 60 nM), being more than 200 times more potent than somatostatin. As measured by the difference between [^{3}H]dihydromorphine, [^{3}H][D-Ala^2,D-Leu^5]enkephalin, and (−)-[^{3}H]bremazocine binding, SMS 201-995 appears to be highly specific for the opiate μ binding site. Electrophysiological data from hippocampal cultures and results from animal studies (tail flick, mydriasis) demonstrate the opiate antagonistic properties of SMS 201-995. SMS 201-995 is an opiate μ antagonist with a peptide structure. That this property is displayed by a somatostatin analog is somewhat unexpected.**

The tetradecapeptide somatostatin has been reported to interact weakly with opiate receptors. In crude rat brain homogenates, only high concentrations of somatostatin displace [^{3}H]-naloxone or [^{3}H]dihydromorphine from their binding sites (1, 2). Its analgesic properties are apparent from results obtained in the tail flick test in mice in which, after intracerebroventricular (i.c.v.) administration, somatostatin produces a naloxone-reversible prolongation of the response latency (2). Furthermore, in the rat, somatostatin (i.c.v. administration) antagonizes the behavioral effects of previously administered β-endorphin (3).

On the basis of these results, it was decided to investigate the possible interactions of the stable and potent somatostatin analog D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-NH-CH(CH_2OH)-$CHOHCH_3$, code named SMS 201-995 (4), with opiate receptors.

MATERIALS AND METHODS

Binding Studies. The brains (minus cerebellum) of male Sprague–Dawley rats and albino guinea pigs were used for binding studies. Preparation of the homogenate and incubation conditions were essentially as described (5).

Nonspecific binding was determined in the presence of 0.1 μM (−)-bremazocine. Radioactive compounds were purchased from New England Nuclear ([^{3}H]naloxone, 18.3 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels; [^{3}H]dihydromorphine, 85.6 Ci/mmol; [D-Ala^2,D-Leu^5] [^{3}H]enkephalin [D-Ala^2,D-Leu^5][^{3}H]EK, 29 Ci/mmol, or labeled in our laboratories [(−)-[^{3}H]bremazocine, 20 Ci/mmol].

Electrophysiology *in Vitro*. Hippocampal cultures were prepared from 4- to 10-day-old rats by the roller-tube technique (6). They were fed at weekly intervals with a medium consisting of 25% horse serum/50% basal medium (Eagle)/25% Hanks' balanced salt solution. For electrophysiological recordings, the cultures were transferred to a microchamber that was continuously perfused with Hanks' balanced salt solution containing the drug being tested.

Reversal of Morphine-Induced Analgesia. The antagonistic effects of SMS 201-995 and naloxone on morphine-induced analgesia [5.6 mg/kg administered subcutaneously (s.c.)] were compared by using the tail flick method in the mouse (7). The antagonists (5–10 animals per dose) were given intravenously (i.v.) (10 ml/kg) or by i.c.v. administration (10 μl/mouse) 2 min before morphine administration (8).

The AD_{50}, estimated graphically according to the method of Litchfield and Wilcoxon (9), was defined as the dose required to reduce, in half of the animals, a 75% increase in the reaction time compared with mean pretreatment control values.

Reversal of Morphine-Induced Mydriasis. In male mice (OF 1 strain), the diameter of the pupil of the right eye of each animal was measured by using a microscope with a graduated eyepiece 15 min before and 29 min after administration of morphine (4.3 mg/kg s.c.). Immediately after the second measurement, each mouse received one dose of antagonist (i.c.v.) and, 5 min later, the pupil diameter was again determined. The change in mean pupil diameter between the values at 29 and 35 min were expressed as a percentage of the pupil diameter at 29 min in the same animal. Five mice were used per dose and the AD_{50} (antagonist dose required to reduce morphine-induced mydriasis by 50% 5 min after its administration) was estimated by regression analysis.

The following drugs were used: naloxone hydrochloride (Endo Laboratories, New York); somatostatin and [D-Ala^2,D-Leu^5]EK (Bachem, Bubendorf, Switzerland); and bremazocine, [D-Ala^2,MePhe^4,-NHCH(CH_2OH)-$(CH_2)_2$-S(O)$CH_3{}^5$]EK (FK 33-824), [D-Ala^2,MePhe^4,-$NHCH_2CH_2OH^5$]EK, and SMS 201-995 (Sandoz).

RESULTS

Ligand Binding Studies. Somatostatin displaced [^{3}H]naloxone in a concentration-dependent manner but only at high concentrations. SMS 201-995 showed a 200-fold greater affinity for the same sites with a Hill coefficient well below unity (Table 1).

To check the specificity of SMS 201-995 for a particular subgroup of opiate receptor sites, binding studies were performed with the μ agonist [^{3}H]dihydromorphine, the δ agonist [D-Ala^2,D-Leu^5][^{3}H]EK, and the κ agonist (−)-[^{3}H]bremazocine (Fig. 1). Whereas low concentrations of SMS 201-995 displaced dihydromorphine (IC_{50}, 23 ± 15 nM) with a Hill coefficient

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Abbreviations: i.c.v., intracerebroventricular(ly); SMS 201-995, D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-NH-CH(CH_2OH)$CHOHCH_3$; [D-Ala^2, D-Leu^5][^{3}H]EK, [D-Ala^2,D-Leu^5][^{3}H]enkephalin; s.c., subcutaneous(ly); i.v., intravenous(ly); FK 33-824 [D-Ala^2,MePhe^4,-NHCH-(CH_2OH)-$(CH_2)_2$-S(O)$CH_3{}^5$]EK; AD_{50}, antagonist dose required to reduce drug-induced effect by 50% 5 min after its administration.

Table 1. Relative potencies of drugs in displacing [^{3}H]naloxone-specific binding to rat brain homogenates

Substance	IC_{50}, nM	Hill coefficient
SMS 201-995	38 ± 60	0.60
Somatostatin	9,000 ± 1,000	0.85
Naloxone	1.8 ± 5	0.95

IC_{50} values for displacement of specifically bound [^{3}H]naloxone were calculated by appropriately weighted regression analysis of Hill plots. The radioactive ligand was used at 1 nM, and mixtures were incubated for 3 hr at 0°C to limit degradation of somatostatin. Similar results were obtained with incubation at ambient temperature in the presence of bacitracin at 50 μg/ml.

close to unity, high concentrations of SMS 201-995 were necessary to displace the δ agonist (IC_{50}, 5,000 ± >1,000 nM) from rat brain membranes. Since [D-Ala^2,D-Leu^5][^{3}H]EK binds not only to the δ- but also to some extent to the μ-opiate site (10), no value for the Hill coefficient was calculated. To occupy μ and δ sites, 1 μM [D-Ala^2, MePhe^4,-NHCH$_2$CH$_2$OH^5]EK/1 μM [D-Ala^2,D-Leu^5]EK was added to guinea pig brain membranes; thus, only displacement of (−)-[^{3}H]bremazocine from the remaining κ sites (10) was studied. SMS 201-995 showed a very weak affinity for opiate κ sites (IC_{50}, 10,000 ± >1,000 nM).

Electrophysiology *in Vitro*. Activity was recorded intracellularly from 15 pyramidal cells in long-term hippocampal explants. Bath application of 10 μM SMS 201-995 increased the firing rate of nine pyramidal cells, inhibited three, and had no effect in three. These effects, the nature of which was not further analyzed, usually subsided after a few minutes, thereby allowing interaction of the peptide with opiates to be tested without a complicating intrinsic activity.

Application of 1 μM FK 33-824, a stable enkephalin analog, induced bursting discharges and paroxysmal depolarization shifts in hippocampal pyramidal cells (Fig. 2*B*), an effect that was naloxone-sensitive and predominantly mediated by opiate μ receptors (11, 12). Bath application of 10 μM somatostatin or 10 μM SMS 201-995 had, 5 min after onset of drug perfusion, no effect on either the excitability of the cell or the synaptic responses (Fig. 2 *C* and *D*). Somatostatin (10 μM) failed to affect the actions of 1 μM FK 33-824 in all four cells tested. In contrast, 10 μM SMS 201-995 prevented the action of 1 μM FK 33-824 in 8 of 10 cells (Fig. 2 *E* and *F*).

Reversal of Morphine-Induced Analgesia. I.c.v. administration of somatostatin in doses up to 0.4 mg/kg did not antagonize the analgesic effect of morphine as measured in the tail flick test in the mouse. SMS 201-995, however, antagonized morphine analgesia after i.c.v. (AD_{50}, 12 μg/kg) or i.v. (AD_{50}, 0.32 mg/kg) administration. This morphine antagonistic effect was about 2–10% that of naloxone (1 μg/kg i.c.v., 7 μg/kg i.v.).

Neither somatostatin nor SMS 201-995 showed any analgesic activity *per se* in the same test system at a series of observation

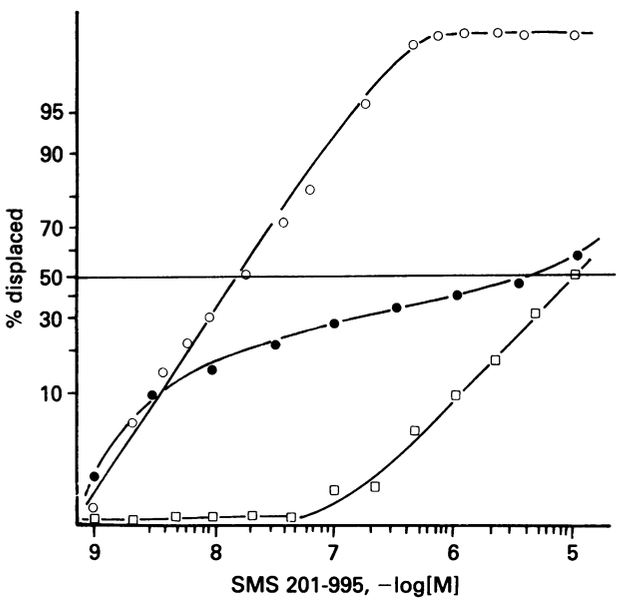

FIG. 1. Displacement of [^{3}H]dihydromorphine (○) and [D-Ala^2,D-Leu^5] [^{3}H]EK (●) from rat brain membranes and (−)-[^{3}H]bremazocine (□) from guinea pig brain membranes by SMS 201-995. Aliquots of homogenate [17 mg of tissue (wet weight) per assay] were incubated for 40 min at room temperature with 0.5 nM [^{3}H]dihydromorphine or 2 nM [D-Ala^2,D-Leu^5][^{3}H]EK and various amounts of displacer. Displacement studies with (−)-[^{3}H]bremazocine (1 nM) were done in the presence of 1 μM [D-Ala^2, MePhe^4,-NHCH$_2$CH$_2$OH^5]EK/1 μM [D-Ala^2,D-Leu^5]EK. Nonspecific binding in the presence of 0.1 μM bremazocine was subtracted from all experimental points. Values are expressed as percentage of labeled ligand specifically displaced and are shown as log–probit plots. The experiment was repeated three times and the results obtained varied by less than 12%.

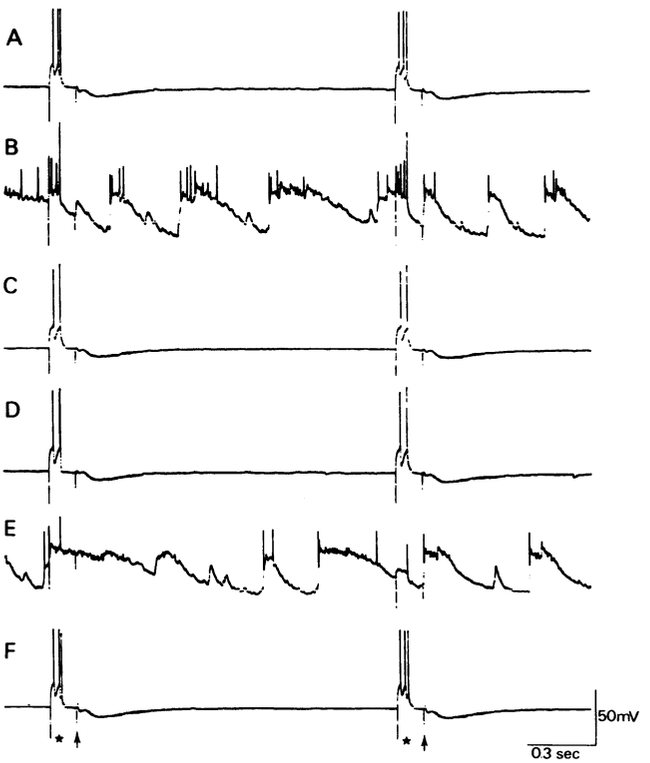

FIG. 2. Opiate antagonistic effect of SMS 201-995 as recorded from hippocampal pyramidal cells. Activity was recorded intracellularly from a pyramidal cell that had been cultured for 49 days. The excitability of the cell was monitored by injection of a depolarizing current pulse through the recording electrode (0.33 Hz, 120 msec, 0.3 nA; indicated in *F* by star). Immediately after the intracellular pulse, field stimulation (0.1 msec, 4 μA; indicated in *F* by arrow) elicited in the pyramidal cell a short latency excitatory postsynaptic potential followed by a long-lasting inhibition (*A*). During application of 1 μM FK 33-824 (*B*), the synaptic responses were transformed into bursting discharges whereby the inhibitory responses were abolished. Bath application of 10 μM somatostatin (*C*) and 10 μM SMS 201-995 (*D*) had, 5 min after onset of the drug perfusion, no effect on either the excitability of the cell or the synaptic responses. In the presence of 10 μM somatostatin, 1 μM FK 33-824 persisted in inducing bursting discharges (*E*) whereas 10 μM SMS 201-995 completely prevented the action of 1 μM FK 33-824 (*F*).

times (2–30 min) by either route of administration.

Reversal of Morphine-Induced Mydriasis. SMS 201-995 (1–10 μg per mouse i.c.v.) and naloxone (0.01–1 μg per mouse i.c.v.) both caused a dose-dependent inhibition of morphine-induced mydriasis with AD_{50} values of 4 and 0.65 μg per mouse. Somatostatin (100 μg per mouse i.c.v.) had no effect on morphine-induced alteration in pupil diameter.

DISCUSSION

The present results show that the somatostatin analog SMS 201-995 binds to opiate receptors in rat brain with about 200 times higher affinity than somatostatin. Electrophysiological experiments *in vitro* indicate that the peptide antagonizes the excitatory effects of a stable enkephalin derivative. Results of animal experiments and data obtained with binding and electrophysiological studies are congruent. Like naloxone, SMS 201-995 antagonized the analgesic and mydriatic effects of morphine in mice. In the tail flick and mydriasis tests, SMS 201-995 was about 10% as potent as naloxone after i.c.v. and i.v. administration.

SMS 201-995 is a conformationally stable cyclic analog of somatostatin that inhibits basal growth hormone secretion in infusion experiments in the rat with an ID_{50} of 0.18 (μg/kg)/hr [somatostatin, 3.5 (μg/kg)/hr]. This effect has been shown to be due to direct interaction with the pituitary (W. Bauer, personal communication). Opiate agonists had no influence on growth hormone release at the pituitary level (13). In rats, experiments with naloxone have yielded ambiguous results; both decrease of growth hormone levels and absence of effects have been reported (14, 15). Possibly the opiate–antagonistic properties of the peptide may contribute to its inhibitory activity on growth hormone secretion.

The preference of SMS 201-995 for the μ-binding site (with a selectivity factor of 200) is of the same order of magnitude as that of specific μ-receptor agonists such as [D-Ala^2, MePhe^4,-$NHCH_2CH_2OH^5$]EK (10) and morphiceptin (16). In view of its specificity, SMS 201-995 may be an interesting tool for neuropharmacological studies, although its affinity for [^{3}H]-dihydromorphine receptor sites is only 10% of that of naloxone.

Electrophysiological recordings from hippocampal pyramidal cells *in vivo* and *in vitro* have shown either an excitatory (17, 18) or an inhibitory action (19) of somatostatin. In accordance with the present results, desensitization usually developed rapidly, so that consecutive applications of the peptide resulted in decreased response. It is to be noted that SMS 201-995 persisted in blocking the opiate effects even after the intrinsic effects of the peptide had subsided.

It was not possible to establish the relative affinities of SMS 201-995 for different types of opiate receptors by using the mouse vas deferens preparation (20) because the compound has an intrinsic inhibiting effect in this tissue that may be related to an action at the somatostatin receptor (21).

A structural relationship between SMS 201-995 and any of the known opioids is not apparent. Although two conformationally constrained enkephalins containing cysteine^2 and cysteine^5 interact with the opiate receptor (22), a molecular interrelationship awaits further clarification.

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