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Differential Effects of Octreotide on Motor Responses to Nicotine in Rats

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WILLIAMS, Y. Z. AND J. D. CONNOR. *Differential effects of octreotide on motor responses to nicotine in rats.* PHARMACOL BIOCHEM BEHAV 43(4) 1165–1168, 1992.—We tested the hypothesis that brain somatostatin levels modify two motor behaviors evoked by ICV infusions of nicotine. Unrestrained, awake rats were given fixed-concentration infusions of nicotine until the prostration/immobility (PI) syndrome and convulsions were produced. Infusion duration ranged from 0.9 to 1.2 min for the PI syndrome and 2.5 to 4.9 min for the convulsions. Octreotide, a stable somatostatin analog (4.5 μg, ICV), significantly raised the threshold for nicotine convulsions 1.0 and 5.5 h after pretreatment but not at 24 or 48 h. Cysteamine, a somatostatin releaser and depletor (0.35–0.75 mg/rat, ICV), also caused a dose-dependent increase in seizure threshold. Similarities in the responses to octreotide and cysteamine suggest that depression of nicotine convulsions by cysteamine may be mediated by release of endogenous somatostatin. Neither octreotide nor cysteamine altered the threshold for the PI syndrome. These results support the view that one motor behavior evoked by nicotine is subject to control by somatostatin whereas another is not.

Somatostatin release Convulsions Prostration/immobility syndrome Cysteamine Nicotinic receptors

NICOTINE by central or peripheral routes of administration causes reproducible motor manifestations in a variety of species. Clonic-tonic convulsions, well-known motor effects of nicotine, are thought to arise by activation of nicotinic cholinergic receptors on brain neurons (5,7). Ganglionic blocking drugs inhibit convulsions (7,16), but atropine does not (1). Brain pathways responsible for initiating nicotine convulsions have not been definitively identified. Nicotine, at doses less than those that elicit convulsions in rats, also causes a prostration/immobility (PI) syndrome characterized by flaccid extension of the limbs (14,26). The syndrome is reported to arise from neurons in the cerebellar nodulus (14). Other, less intense motor behaviors induced by nicotine may originate with basal ganglia neurons, in particular those in the nucleus accumbens (18,30).

Somatostatin-14, a tetradecapeptide present in the brain and other tissues, influences motor activities (24) and can evoke barrel-rotation seizures in rats (9,14). Nicotine given intravenously increases somatostatin-like immunoreactivity and upregulates somatostatin binding (4). Alternatively, somatostatin is depleted from brain and other tissues by systemic injection of cysteamine (25) by a mechanism involving peptide release from tissue stores (3).

Because somatostatin and nicotine both exert profound effects on motor behaviors and apparently interact at the neuronal receptor level, we studied nicotine convulsions and the PI syndrome in rats injected ICV with octreotide (SMS 201-995), a more stable somatostatin analog (22), or cysteamine. The question was whether either of the motor effects of nicotine cited would respond to changes in somatostatin activities in the brain.

METHOD

Subjects

Male Sprague-Dawley rats (CRBL, 200–400 g) were housed under controlled illumination (12-h cycles) and temperatures (21–23°C). The 126 rats used in this study were given free access to a standard rat chow and water.

Drugs and Drug Administration

All drugs except anesthetics were injected directly into the left lateral cerebroventricle to minimize peripheral actions (nicotine, cysteamine) or ensure delivery of a known quantity into the brain (octreotide). Infusions were utilized rather than

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bolus injections because infusions allow for slower, but controlled, development of motor behaviors evoked by nicotine. Onset times for the motor end points provide an index of total threshold doses needed for the responses. (*S*)-(-)-nicotine (free alkaloid), 6.2 mM, was infused at 16 μl/min until the motor end points were observed. Octreotide was injected at 16 μl/min for 1.5 min; two doses were delivered by infusing two concentrations (47 or 210 mM). Cysteamine (650 mM) was infused at 1.5 μl/min; the dose was regulated by altering the duration of infusion. The drugs were dissolved in artificial cerebrospinal fluid (aCSF) composed of (mM/l): Na^+ 141, K^+ 3.3, CA^{++} 1.25, Mg^{++} 1.2, Cl^- 152, $H_2PO_4^-$ 0.48, HCO_3^- 21, glucose 3.34, and urea 2.2. Control rats were given aCSF alone using the same volumes and rates of infusion as the corresponding experimental group. (−)Nicotine and cysteamine were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI) and Sigma Chemical Co. (St. Louis, MO), respectively. Octreotide (SMS 201-995) was a gift from Sandoz Pharmaceuticals.

Surgery

Rats were anesthetized with sodium pentobarbital (45 mg/kg, IP) and ketamine (10 mg/kg, IM) and then placed in a stereotaxic instrument with the top of the head horizontal. The skull was exposed by midline incision, skin and muscle retraction, and removal of the periosteum. A 2-mm diameter hole was made in the skull 1 mm posterior to the bregma and 1 mm lateral to the midline. A silastic-filled guide cannula fashioned from a 20-ga hypodermic needle (28) was advanced 4.5 mm from the top of the skull so that its tip was in the left lateral cerebroventricle near the dorsal hippocampus (20). A small stainless steel screw was driven more anteriorly to anchor a layer of acrylic cement to the skull.

Procedure

Three days after surgery, motor behaviors were studied in a quiet setting under low light using awake, unrestrained rats. An injection needle (25 ga) was inserted through the silastic seal of the guide cannula. Solutions were delivered through PE 50 tubing from a 1-ml syringe in a Harvard infusion pump (Bard MedSystems, North Reading, MA). Clear plastic cages allowed visual monitoring of animal behavior. Nicotine was infused at scheduled times after octreotide, cysteamine, or aCSF alone. Latencies to the onset of the PI syndrome and convulsions were measured with a stopwatch. The PI syndrome was defined as flaccid paralysis of the limbs with tail extension and the head resting on the bedding in the cage. The moment of head drop was taken as the end point. The onset of convulsions was defined by a whole-body clonic twitch that moved the rat upward and backward. At the end of the experiments, rats were anesthetized with barbiturate, decapitated, and injected through the guide cannulae with methylene blue. The few rats that showed nonuniform dye distribution in the ventricular spaces were excluded from the study. Data were analyzed statistically using a pooled *t*-test (10) with $p < 0.05$ considered significant.

RESULTS

PI Syndrome

aCSF infusions alone (25 μl, ICV, over 1.5 min) did not elicit obvious changes in motor or open-field behaviors in awake, unrestrained rats. However, in rats infused 4 h previously with aCSF nicotine ICV caused the PI syndrome at a mean (±SEM) latency of 1.19 (±0.11) min. The syndrome occurred at 1.26 (±0.13) min in rats pretreated with octreotide (4.5 μg, ICV, 4 h previously) and at 1.17 (±0.05) min in

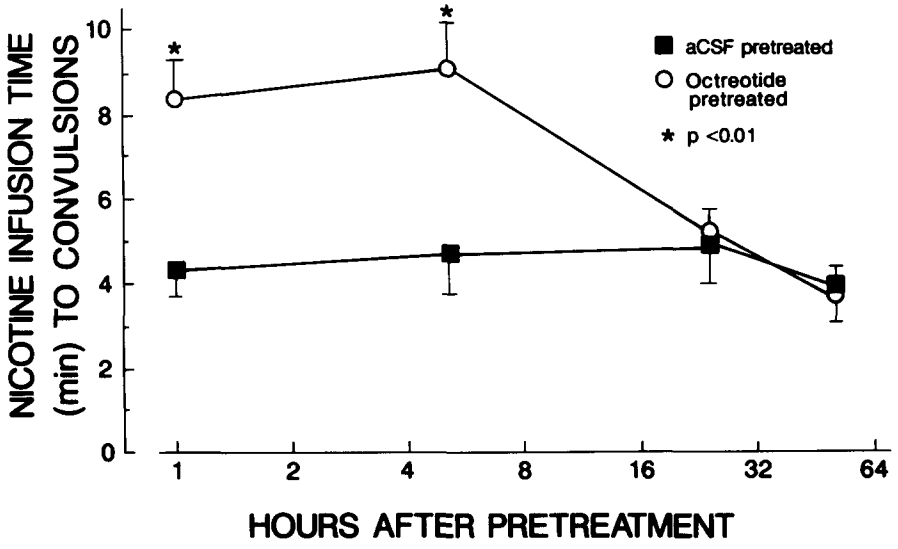

FIG. 1. Time course for depression by octreotide of nicotine-induced convulsions. Octreotide, total dose 4.5 μg, or artificial cerebrospinal fluid (aCSF) was infused ICV 1.0, 5.5, 24, and 48 h before nicotine. (−) Nicotine, 6.2 mM solution, was infused ICV until the clonic end point. Significant differnces between aCSF and octreotide pretreatments are indicated with an asterisk (n = 5–15 rats/mean).

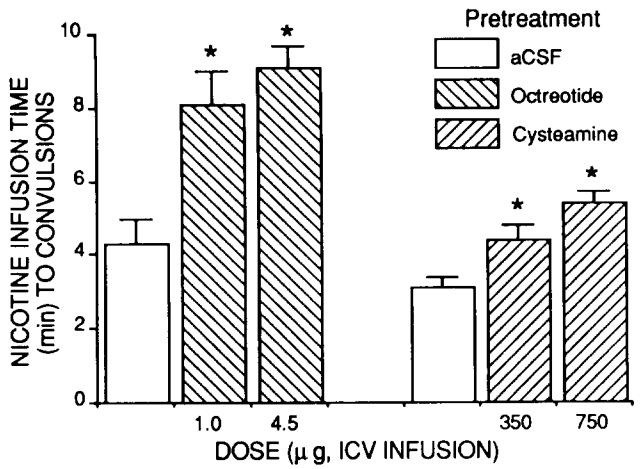

FIG. 2. Comparison of depressant effects of octreotide and cysteamine on nicotine-induced convulsions. Artificial cerebrospinal fluid (aCSF) or one of two doses of octreotide or cysteamine was infused ICV 5 h before nicotine. (−) Nicotine, 6.2 mM solution, was infused ICV until the clonic end point. Significant differences relative to controls are indicated with an asterisk ($p < 0.02$ for octreotide; $p < 0.05$ for cysteamine). The bars represent means ± SEM for $n = 6$ rats/mean.

rats pretreated with cysteamine (750 μg, ICV, 4 h previously). Differences in latency to onset of the PI syndrome in treated and control groups were not statistically significant.

The rates of clearance of octreotide and cysteamine from rat brain after ICV injection are unknown. However, we found that up to 5.5 h postinjection octreotide had a major effect on nicotine convulsions (Fig. 1). A similar time course for anticonvulsant activity was obtained for cysteamine. The 4-h interval between pretreatment and nicotine challenge diminishes the likelihood that physical interactions between the drugs at the ventricular site of introduction could be responsible for a lack of effect on behavior.

Convulsions

Time-action and dose-response results after octreotide pretreatments revealed that the octapeptide significantly raised the threshold for nicotine convulsions. Change in seizure threshold was measured as an increase in the duration of nicotine infusion needed to elicit the clonic convulsion end point (longer latency to onset).

Figure 1 shows the time course of the convulsive effect of nicotine infused 1.0, 5.5, 24, or 48 h after a fixed infusion of octreotide (45 μg, ICV, total dose). The latency to convulsive seizures was significantly prolonged at 1.0 and 5.5 h after octreotide; the doses of nicotine required for convulsions were approximately double those of rats pretreated with aCSF. Octreotide effects diminished to control levels between 5.5 and 24 h postinfusion.

The anticonvulsant effects of two levels of pretreatment with octreotide or cysteamine relative to aCSF are shown in Fig. 2. In rats given 25 μl aCSF ICV 5 h previously, convulsions were produced by nicotine at a mean (±SEM) latency of 2.4 (±0.6) min ($n = 6$ rats) and 3.2 (±0.3) min for a different group of rats ($n = 7$). Significant increases in nicotine required for convulsive seizures were obtained in rats pretreated with octreotide or cysteamine (Fig. 2). The duration of nicotine infusions to attain the clonic end point was approximately doubled by the two doses of octreotide and was increased about 30% by 350 μg ICV cysteamine.

DISCUSSION

Inhibition of nicotine seizures by a somatostatin analog was an unexpected observation. Other investigators have shown that somatostatin enhances excitatory cholinergic responses in the rat hippocampus (15), augments the spread of limbic seizures (21), and causes vestibular "barrel rolling" (9,14). Proven interactions between somatostatin and nicotine that might account for inhibition of convulsions are sparse. Somatostatin inhibits nicotinic receptor responses in chromaffin cells by facilitating receptor desensitization or by open-channel block (12). If a similar interaction were to occur in a seizure-sensitive brain area such as the hippocampal formation, the peptide might counteract local effects of nicotine. Somatostatin promotes acetylcholine release from rat hippocampal slices (2), a process that might lead to receptor desensitization.

High concentrations of somatostatins and intermediate to high densities of somatostatin receptors have been reported in the hippocampal neuropil (6,13,23). Moreover, nicotinic (8) and muscarinic (27) binding sites have been identified in this brain region. However, field potentials from CA1 pyramidal cells evoked by activation of nicotinic receptors are blocked by mecamylamine, but not other nicotinic antagonists, suggesting that the receptors may be subtype variants of the traditional nicotinic receptors (11).

The design of our experiments was such that particular brain regions or pathways responsible for drug effects cannot be discerned. It remains a distinct possibility that mechanisms for nicotine and somatostatin are not microanatomically coupled, that is, the compounds may have sites of action on different neurons whose outputs converge at a more distant nexus. Convulsions are complex motor behaviors that presumably can be generated and antagonized at numerous points in the nervous system.

Cysteamine releases and depletes somatostatin (3,25). Relative selectivity of these effects compared to effects on other neuropeptides (19) has led to the use of cysteamine in studies of the functional roles of somatostatin in the brain (17,29). In our experiments, octreotide and cysteamine given ICV both depressed nicotine convulsions. A parsimonious explanation would be that cysteamine releases endogenous somatostatin in amounts and for durations sufficient to counteract nicotine convulsions.

Neither octreotide nor cysteamine affected the PI syndrome induced by nicotine. The syndrome is reported to originate with activation of neurons in the cerebellar cortex (14). Although the syndrome was once considered a noncholinergic response, the signs are blocked by mecamylamine and hexamethonium (26). Our results indicate that neural mechanisms that give rise to the syndrome, unlike those responsible for nicotine convulsions, are independent of somatostatin modulation.

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