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BRIEF COMMUNICATION

Cocaine Produces Cholinergically Mediated Analeptic and EEG Arousal Effects in Rabbits and Rats

M. YABASE,[1] M. A. CARINO AND A. HORITA[2]

Departments of Pharmacology and Psychiatry & Behavioral Sciences
University of Washington School of Medicine, Seattle, WA 98195

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YABASE, M., M. A. CARINO AND A. HORITA. *Cocaine produces cholinergically mediated analeptic and EEG arousal effects in rabbits and rats*. PHARMACOL BIOCHEM BEHAV **37**(2) 375–377, 1990.—Cocaine (1–5 mg/kg, IV) shortened the duration of loss of righting reflex produced in pentobarbital-narcotized rabbits. This effect was completely blocked by scopolamine (1 mg/kg, IV), but not by scopolamine methylbromide, suggesting that a central cholinergic mechanism was involved. In urethane-anesthetized rats cocaine (1 mg/kg, IV) consistently generated hippocampal EEG theta rhythm lasting about 40 min. This effect was also abolished by scopolamine. These results suggest that cocaine produced behavioral and EEG arousal responses that involved the septohippocampal cholinergic system.

Cocaine Cholinergic Analeptic EEG arousal Hippocampus

IN addition to its local anesthetic, psychomotor stimulant and drug abuse properties, cocaine possesses a less well-known analeptic effect in drug narcotized animals (13). The mechanism of this analeptic effect is not known.

During the past ten years we have found that several peptides and drugs that exert analeptic activity do so by activating central cholinergic arousal systems in hippocampus and cortex. In the present paper we report on the cholinergic nature of cocaine-induced analepsis in rabbits and hippocampal EEG arousal in rats.

METHOD

Male New Zealand rabbits, weighing 2.3–2.7 kg (R & R Rabbitry, Stanwood, WA), and male Sprague-Dawley rats, 300–350 g (Tyler Labs) were used in these studies. All experiments were conducted in an ambient temperature of 22.0 ± 1.0°C.

The following drugs, the doses of which are expressed as the base, were dissolved in 0.9% saline solution: cocaine HCl; pentobarbital Na; scopolamine hydrobromide; scopolamine methyl bromide. All injections in rabbits were made into a cannulated marginal ear vein. In the EEG studies urethane was administered IP, but all other drug injections were made intravenously via a cannulated external jugular vein. Drug injections were given in volumes of 0.1 ml and washed in with another 0.1 ml normal saline.

The analeptic property of cocaine was measured as a shortening of the duration (recovery of the righting reflex) of narcosis produced by pentobarbital. Integrity of the righting reflex was determined by placing the animal on its back and observing if it would resume and maintain the upright position. Injections and observations of animals were made by different individuals; i.e., the observer was not aware of the treatments given to each animal.

EEG activity was recorded from the right hippocampus of rats anesthetized with urethane (1.25 g/kg, IP). Urethane was used in the EEG studies because of its suitability as a general anesthetic for studies of neural functions (7). Supplemental doses were given when needed to maintain surgical anesthesia. The trachea was cannulated to facilitate breathing and the jugular vein cannulated for drug injections. The rat was then placed in a stereotaxic apparatus with bregma and lambda at the same horizontal level. A concentric bipolar stainless steel recording electrode (NEX-100, Rhodes Medical) was lowered into the dorsal hippocampus (3.5 mm posterior to bregma, 1.8 mm lateral to midline, and 2.8 mm below the dural surface) via a hole drilled in the skull. The signal was led into amplifiers with ½ amplitude low and high filter

[1]Permanent Address: Department of Psychiatry, Tokyo Medical College, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo, Japan.
[2]Requests for reprints should be addressed to A. Horita, Department of Pharmacology SJ-30, University of Washington Medical Center, Seattle, WA 98195.

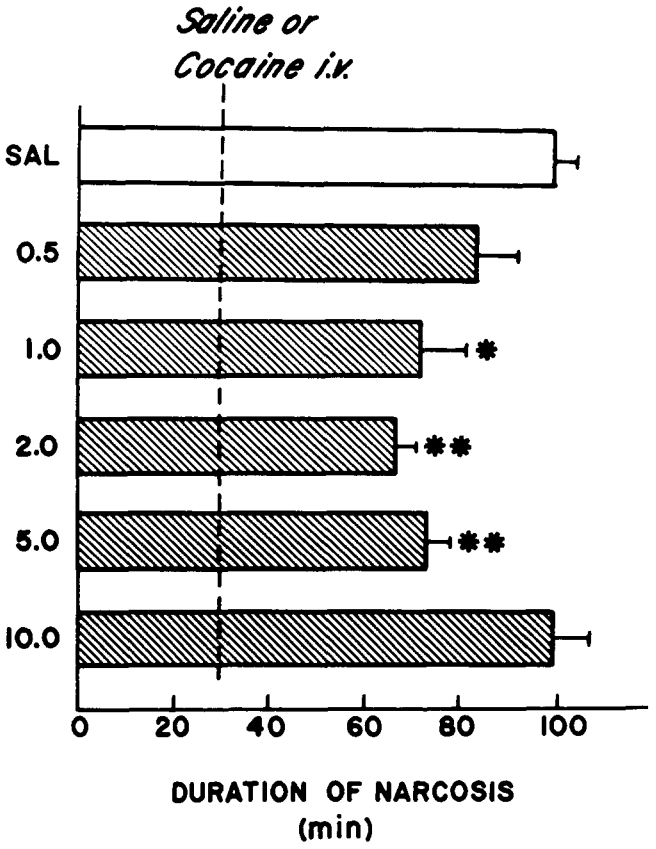

FIG. 1. Effect of varying doses (mg/kg IV) of cocaine on the duration of pentobarbital-induced narcosis in rabbits. Each bar represents the duration (mean ± SEM) of narcosis under the drug conditions described in 6 to 8 animals. Cocaine was given 30 min after pentobarbital (30 mg/kg IV) administration. *$p<0.05$ when the 1 mg/kg cocaine animals were compared with the saline group, and **$p<0.01$ when the 2 and 5 mg/kg cocaine animals were compared with the saline group.

settings at 1 and 15 Hz, respectively. The signal was displayed on paper using a Grass polygraph. Hippocampal EEG activity was analyzed visually, and theta activity was identified as rhythmic high-voltage sinusoidal-like waves having a frequency of 3–10 Hz (1). Core temperature was maintained at 35.0 ± 0.5°C by means of a thermostatically controlled heating lamp.

The data were analyzed by one-way analysis of variance, and the differences between treatment groups compared by the Newman-Keuls method. A difference of $p<0.05$ was considered statistically significant.

RESULTS

The analeptic effect of varying doses of cocaine in pentobarbital-narcotized rabbits is presented in Fig. 1. One-way analysis of variance indicated a significant difference among the treatment groups, $F(5,35)=5.2$, $p<0.005$. Newman-Keuls test showed that the duration of narcosis in animals treated with 1, 2 or 5 mg/kg cocaine were significantly shorter than saline-treated animals. The 0.5 mg/kg dose also produced shortening of narcosis but did not reach statistical significance. The highest dose of cocaine used (10 mg/kg) was totally devoid of analeptic activity.

The cholinergic nature of the analeptic effect is shown in Table 1. The 2 mg/kg dose of cocaine was used to demonstrate

TABLE 1

ANALEPTIC EFFECT OF COCAINE (2 mg/kg IV) IN PENTOBARBITAL-NARCOTIZED RABBITS AND ITS REVERSAL BY SCOPOLAMINE (1 mg/kg IV)

	Drug Treatment			
1	2	3	N	Mean Duration of Narcosis (min ± S.E.M.)
A) PB	Saline	Saline	8	99 ± 5
B) PB	Saline	Cocaine	8	67 ± 4*
C) PB	Scop	Saline	5	102 ± 8
D) PB	Scop	Cocaine	6	97 ± 5†
E) PB	ScopMeBr	Cocaine	6	62 ± 5

PB = Pentobarbital, 30 mg/kg IV; Scop = scopolamine; ScopMeBr = scopolamine methylbromide, 1 mg/kg IV.

One-way analysis of variance showed differences among the treatment groups, $F(4,27)=12.2$, $p<0.005$.

*$p<0.001$ when treatment B is compared to treatment A; †$p<0.001$ when treatment D is compared to treatment B, as determined by Newman-Keuls test.

Drug 2 was administered 10 min, and drug 3, 30 min, after drug 1.

shortening of pentobarbital narcosis. While scopolamine (1 mg/kg, IV) alone did not affect pentobarbital narcosis, it completely blocked the analeptic effect of cocaine ($p<0.001$). In contrast, scopolamine methylbromide, and quaternary analog of scopolamine, was inactive in reversing the analeptic effect, indicating that a central cholinergic mechanism mediated the cocaine-induced analeptic effect. Although not reported here, atropine also produced blockade of the cocaine-induced analeptic effect.

Cocaine also produced EEG arousal in urethane-anesthetized rats. We employed the rat because our familiarity with the arousal EEG of this species from past studies (2,4) and because analeptic activity in rats is much more difficult to demonstrate. Hippocampal EEG measurements showed that cocaine, 1 mg/kg IV, generated a theta rhythm as shown in Fig. 2. Doses as low as 0.1 mg/kg of cocaine produced hippocampal theta, but was not a consistent response at this low dose. Peak response appeared 5–10 min after cocaine administration and persisted for 20–30 min, after which the response subsided over the following 10–20 min. We also employed a dose of 10 mg/kg IV cocaine to rats, but this dose

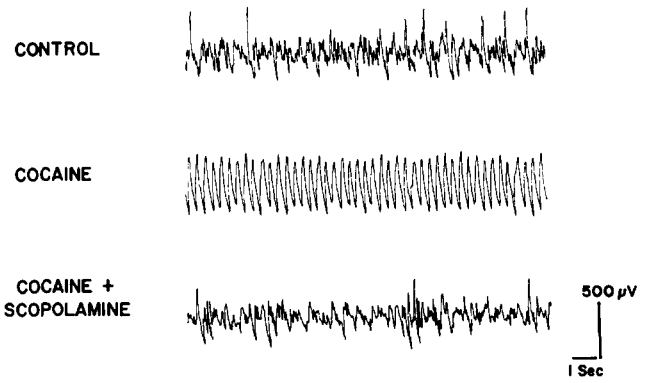

FIG. 2. Hippocampal theta generating effect of IV cocaine and its blockade by scopolamine in rats. Top panel shows nonrhythmic activity under control (saline) conditions. Middle panel shows theta rhythm 10 min after IV cocaine (1 mg/kg IV). Bottom panel shows abolition of theta activity by scopolamine (1 mg/kg IV) given 15 min after cocaine.

proved lethal in 4 of 6 animals, even when the drug was infused slowly. Administration of scopolamine (1 mg/kg IV) during the peak cocaine response completely abolished theta activity for the remainder of the duration of cocaine action.

DISCUSSION

We have demonstrated that the analeptic and hippocampal EEG effects of cocaine are both blocked by scopolamine, but not by its quaternary analog, suggesting that they were mediated by central cholinergic mechanisms. To our knowledge, this is the first demonstration that cocaine activates central cholinergic mechanisms in producing part of its pharmacological responses. Robinson and Hambrecht (11) demonstrated that a high dose of cocaine (30 mg/kg IP) increased ACh turnover in hippocampus in conscious rats. Other references have suggested anticholinergic properties of cocaine because of its close structural similarity to atropine, among other reasons (9). High concentrations of cocaine block acetylcholine-induced smooth muscle contractions (12), which might explain why only the lower doses (2 and 5 mg/kg) of cocaine were analeptic. The higher dose (10 mg/kg) might have exerted sufficient anticholinergic activity to block the cholinergically mediated analeptic effect. We could not test this idea on the rat EEG because of the lethal nature of 10 mg/kg cocaine.

The mechanism of cocaine induced analepsis is not understood. It is probable that like amphetamine, cocaine produces cholinergic activation via a catecholaminergic mechanism. Pepeu and coworkers (8,10) have shown conclusively that amphetamine causes increased acetylcholine release in hippocampus and cortex via a dopaminergic and/or noradrenergic mechanism. As with cocaine, amphetamine-induced analepsis and hippocampal theta may represent pharmacological responses mediated by the increased acetylcholine release since it is also blocked by anticholinergic drugs (5). Moreover, it has been demonstrated repeatedly that hippocampal and/or cortical cholinergic activation are associated with the analeptic response (2–4, 6).

The role of increased acetylcholine release in the behavioral actions of cocaine is not clear. Perhaps the cholinergically mediated arousal might be responsible for the well-known property of insomnia produced by the drug.

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