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Codeine Produces a Cholinergically Mediated Analeptic Effect in Rats and Rabbits

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HORITA, A., M. A. CARINO AND C. CHINN. *Codeine produces a cholinergically mediated analeptic effect in rats and rabbits*. PHARMACOL BIOCHEM BEHAV **30**(1) 115–118, 1988.—The intravenous administration of codeine to diazepam-narcotized rabbits resulted in a shortened duration of loss of righting reflex. Coadministration of naltrexone plus codeine enhanced this analeptic effect and was also effective in shortening the duration of pentobarbital narcosis. The analeptic effect was blocked by atropine, but not by methylatropine, indicating involvement of a central cholinergic mechanism. In rats the analeptic activity correlated with the reversal of the diazepam-induced fall in sodium dependent high affinity choline uptake in hippocampal and cortical synaptosomes. These findings may represent the pharmacological basis of the recently reported antinarcoleptic action of codeine in man.

Codeine Analepsis Diazepam Narcosis Cholinergic

THE complexity of the actions of the opiates on the central nervous system is well known. In addition to their characteristic production of analgesia, sedation, respiratory depression and euphoria, some of the opiates also produce behavioral excitation when administered in large doses. In several species of animals, stimulation rather than sedation is the predominant effect. Jacquet and Lajtha [8] observed what they described as "explosive" motor behavior when morphine was injected into the periaqueductal gray areas of mice. In the rabbit, as in man, most opiates produce sedation, analgesia, hypothermia and respiratory depression. However, when injected into the cerebral ventricles of rabbits, 25–100 μg of morphine produced gradual hyperthermia, catalepsy, rigidity and hyperreflexia; at 250–500 μg, it produced extreme hyperthermia and excitement [1].

We recently reported on an analeptic (arousal) property of morphine when it was administered by the intracerebroventricular (ICV) route to rabbits anesthetized with pentobarbital [6]. This effect was also produced when morphine was administered intravenously, but only when the animals were pretreated with naloxone or naltrexone. In a more recent study we evaluated a number of opiate agents to determine whether this analeptic effect was mediated by a specific subtype of the opiate receptor [18]. Among several of the compounds that showed antipentobarbital effects when administered ICV, codeine was of interest, especially because of the recent report that it was effective in reversing sleep disorder in the human narcoleptic [5]. We therefore undertook the present study to characterize in greater detail the analeptic property of codeine in the rabbit and rat.

METHOD

Animals

Male New Zealand rabbits, weighing 2.3–2.7 kg (R & R Rabbitry, Stanwood, Washington), 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.

Drugs

The following drugs, the doses of which are expressed as the base, were dissolved in 0.9% saline solution: codeine phosphate; naltrexone HCl (E. I. duPont deNemours & Co.); ketamine; pentobarbital Na; atropine sulfate and atropine methyl bromide. The injectable form of diazepam (Roche) was used to avoid difficulties in solubility of this drug. All injections in rabbits were made into a cannulated marginal ear vein, whereas the IP route was used in rats.

Analeptic Activity

The arousal property of codeine was measured as a shortening of the duration (recovery of the righting reflex) of narcosis produced by the depressant drugs. 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.

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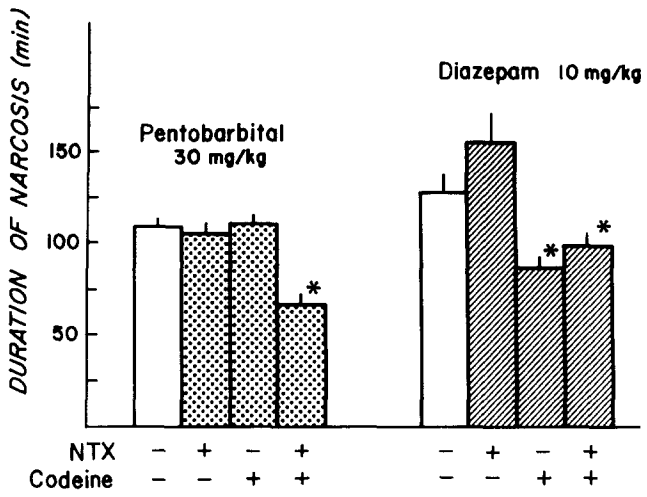

FIG. 1. Effect of codeine (2.0 mg/kg, IV) on the duration of pentobarbital- and diazepam-induced narcosis in rabbits in the presence and absence of naltrexone (NTX, 0.1 mg/kg, IP) pretreatment. Each bar represents the duration (mean±S.E.M.) of loss of righting reflex under various drug conditions in 6 to 12 animals. Naltrexone was given 10 min prior to, and codeine 10 min after, pentobarbital or diazepam administration. *$p<0.01$ as compared to pentobarbital or diazepam controls.

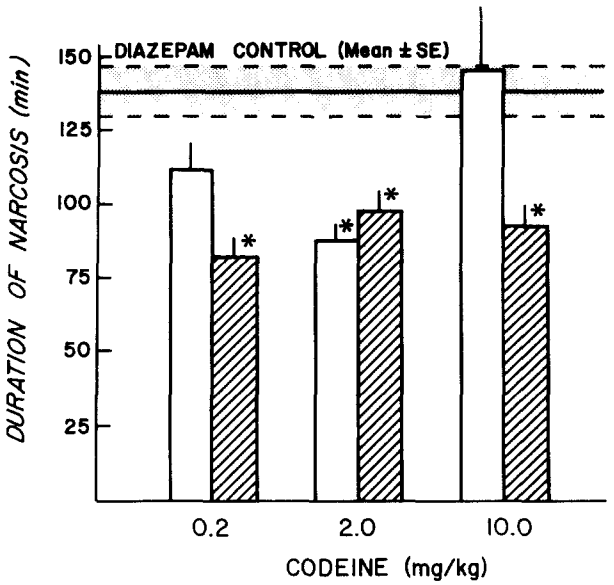

FIG. 2. Effect of varying doses of codeine on duration of diazepam-induced narcosis in rabbits in the absence (clear bars) and presence (diagonal bars) of naltrexone pretreatment. Each bar represents the duration (mean±S.E.M.) of loss of righting reflex in 6 to 19 animals. Naltrexone (0.25 mg/kg, IV) was given 10 min prior to, and codeine 10 min after, diazepam administration. *$p<0.01$ as compared to diazepam control.

High Affinity Choline Uptake (HACU)

The method employed was based on the procedure described by Yamamura and Snyder [17] and modified by Zucker *et al.* [19]. Synaptosomes of hippocampus or frontal cortex were prepared and incubated with 5 μM choline containing 0.4 μCi of ^{3}H-choline chloride (80 Ci/mmol), New England Nuclear. The incubation was started by transfer of the samples from an ice bath to a water incubator at 38°C. After 4 min of incubation, the uptake reaction was terminated by transfer back to the ice bath. The particulate fraction in each sample was collected by centrifugation at 8000 × g for 20 min. The remaining medium was decanted and the pellet surface washed with 1 ml of ice-cold 0.9% saline. After removal of the saline, the pellet was dissolved overnight with 0.7 ml Protosol (NEN). All uptake experiments were performed in triplicate and radioactivity determined by liquid scintillation technique. Protein concentration was measured by the method of Lowry *et al.* [11] with bovine serum albumin as external standard. Sodium-dependent HACU was expressed as pmol choline uptake/mg protein/4 min.

Data Analysis

The data were analyzed employing Student's *t*-test or 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

Analeptic Effect of Codeine in Rabbits

As shown in Fig. 1 the duration of pentobarbital-induced narcosis was not affected by 2.0 mg/kg IV of codeine. Doses of 0.5, 1.0, 2.0, 5.0 and 10.0 mg/kg of codeine were tried, but these were also ineffective in restoring the righting reflex of these pentobarbitalized animals. However, if given to rabbits that had been pretreated with 0.1 mg/kg of naltrexone, codeine (2.0 mg/kg) significantly shortened the duration of narcosis ($p<0.01$) produced by subsequent pentobarbital administration. Although not shown here, larger doses of codeine in the presence of naltrexone did not further shorten the narcosis duration. In contrast, the duration of narcosis produced by diazepam was clearly reversed by codeine, with or without naltrexone pretreatment (Fig. 1). A similar analeptic effect of codeine was observed against the anesthesia produced by 60 mg/kg of ketamine (not shown).

Drug Interaction Effects on Codeine Analepsis

In order to determine whether the analeptic effect of codeine was mediated via classical opiate receptor mechanisms and/or cholinergic mechanisms as we reported earlier for morphine, we conducted interaction experiments with naltrexone and with anticholinergic drugs on the analeptic effect of codeine against diazepam-induced narcosis. The effect of naltrexone on the analeptic action of three different doses of codeine is shown in Fig. 2. Codeine at 2.0 mg/kg, but not at 0.2 mg/kg or 10.0 mg/kg, significantly reduced duration of diazepam-induced narcosis ($p<0.01$). Naltrexone (0.25 mg/kg, IV) by itself exerted no influence on diazepam-induced narcosis. However, it unmasked the analeptic effect of the low (0.2 mg/kg) and high (10 mg/kg) doses of codeine, but did not further enhance the analeptic effect of 2 mg/kg codeine. The effect of atropine and atropine methyl bromide on the codeine effect is shown in Table 1. Analysis of variance showed significant differences among treated and control groups, $F(5,42)=10.5$, $p<0.001$. Atropine completely blocked the analeptic effect of codeine, whereas methylatropine did not, indicating that the effect was mediated via

TABLE 1

EFFECT OF 2.0 mg/kg OF ATROPINE OR ATROPINE METHYL-BROMIDE (METHATROPINE) ON THE ANALEPTIC ACTION OF CODEINE (2.0 mg/kg, IV)

Drug Treatment	N	Mean Duration of Narcosis (min ± S.E.M.)
Diazepam + Saline	12	128 ± 9
Diazepam + Codeine	12	86 ± 6*
Atropine + Diazepam + Saline	7	156 ± 12
Atropine + Diazepam + Codeine	8	140 ± 11
Methatropine + Diazepam + Saline	4	149 ± 9
Methatropine + Diazepam + Codeine	5	89 ± 6*†

Rabbits were treated with anticholinergic drugs 10 min prior to, and codeine 10 min after, the administration of diazepam (10 mg/kg, IV).

One way analysis of variance showed differences among the treatment groups, $F(5,42)=10.5$, $p<0.001$.

*$p<0.05$ as compared to Diazepam + Saline.

†$p<0.01$ as compared to Atropine + Diazepam + Codeine.

TABLE 2

EFFECT OF CODEINE (0.2 mg/kg, IP) ON THE DURATION OF DIAZEPAM-INDUCED NARCOSIS IN RATS

Drug Treatment	N	Mean Duration of Narcosis (min ± S.E.M.)
Naltrexone + Diazepam + Saline	6	204 ± 21
Naltrexone + Diazepam + Codeine	7	114 ± 15*

Rats were treated with naltrexone (0.1 mg/kg, IP) 5 min prior to, and codeine 15 min after, the administration of diazepam (25 mg/kg, IP).

*$p<0.005$ as compared to Naltrexone + Diazepam + Saline.

TABLE 3

EFFECT OF CODEINE (0.2 mg/kg, IP) ON THE DIAZEPAM-INDUCED DECREASE IN HACU ACTIVITY IN RAT HIPPOCAMPAL AND CORTICAL SYNAPTOSOMES

	Drug Treatment			HACU (pmol/mg protein/ 4 min ± S.E.M.)	
	1	2	3	Cortex	Hippocampus
A.	Saline	Saline	Saline	29 ± 1(5)	28 ± 1(5)
B.	Saline	DZP	Saline	18 ± 2(5)*	20 ± 2(5)†
C.	Saline	DZP	Codeine	19 ± 2(5)*	20 ± 2(5)†
D.	NTX	Saline	Codeine	25 ± 1(4)	29 ± 1(4)
E.	NTX	DZP	Saline	22 ± 2(7)	20 ± 1(9)†
F.	NTX	DZP	Codeine	29 ± 3(7)	27 ± 1(8)‡

Drugs 2 and 3 were administered IP 5 and 20 min, respectively, after drug 1. The animals were sacrificed 60 min after drug 3, the brains removed and synaptosomes prepared.

NTX = naltrexone (0.1 mg/kg, IP); DZP = diazepam (25 mg/kg, IP). One way analysis of variance showed significant differences in synaptosomal HACU activities of cortex, $F(5,28)=7.5$, $p<0.005$, and hippocampus, $F(5,30)=9.3$, $p<0.005$, with the different treatments. NTX or codeine controls showed no effect on HACU (NTX—cortex 32 ± 2, hippocampus 28 ± 3; codeine—cortex 30 ± 2, hippocampus 27 ± 2 compared to saline control (A). HACU units expressed as above in Table 2B). Figures in parentheses indicate number of animals.

*$p<0.05$ when treatment B or C is compared to treatment A.

†$p<0.01$ when treatment B, C or E is compared to treatment A.

‡$p<0.01$ when treatment F is compared to treatment E.

central cholinergic mechanisms. Although the data are not shown here, we have demonstrated antagonism of the codeine-induced analepsis of pentobarbital narcosis by atropine, much like our earlier observations with morphine [6].

Codeine Analepsis in Rat and Its Relationship to HACU in Brain Synaptosomes

The analeptic effect of codeine was also demonstrable in the rat. As shown in Table 2 codeine (0.2 mg/kg, IP), in the presence of naltrexone (0.1 mg/kg, IP), reduced the duration of loss of righting reflex from 204±21 min to 114±15 min ($p<0.005$) after diazepam (25 mg/kg, IP) administration.

The effect of diazepam and diazepam + codeine in the absence and presence of naltrexone on HACU is shown in Table 3. Naltrexone, codeine, or the combination of the two (treatment D, Table 3) administered to control animals had no effect on HACU as compared to saline controls. Diazepam lowered HACU in cortex and hippocampi of saline-pretreated rats by 37.9% and 28.6%, respectively. Hippocampal, but not cortical, HACU of naltrexone-pretreated rats also showed significant decreases after diazepam treatment. Codeine administered to diazepam-narcotized rats reversed the depressed HACU activity in hippocampus to normal levels, but only in the presence of naltrexone (E vs. F, Table 3). Cortical synaptosomes from naltrexone-diazepam treated animals also showed a similar restoration of HACU activity, but this increase did not reach statistical significance.

DISCUSSION

As in our earlier findings with morphine, we found that codeine also exerted an analeptic effect in rabbits narcotized with depressant drugs. However, there was a notable difference between the morphine- and codeine-induced analeptic effects. Codeine did not produce a lethal interaction with pentobarbital and was effective as an analeptic against diazepam narcosis even in the absence of naltrexone pretreatment. When morphine was used intravenously it was always necessary to pretreat animals with naloxone or naltrexone to prevent the lethal respiratory depression produced by the drug combination, and only under that condition was it possible to demonstrate the analeptic effect [6].

With codeine there appeared to be an optimal window in the mid-dose range tested (2.0 mg/kg) where naltrexone did not further enhance the analeptic effect. With lower or higher doses of codeine the analeptic response was absent, and only after pretreatment with naltrexone did it become

apparent. The reason for this "window effect" at the mid-dose level is not clear from our present study.

The analeptic action of codeine appears to involve a cholinergic mechanism since atropine blocked the response, much like our earlier observations with morphine antagonism of pentobarbital narcosis [6]. Although we have not yet established the neural substrate of this effect, we may assume that hippocampal and cortical cholinergic neurons are involved, especially since hippocampal and cortical HACU activation were produced by codeine in diazepam-narcotized rats. In these respects the analeptic effect of codeine resembles closely the effect produced by TRH in narcotized animals [10]. In addition to arousing animals from narcosis, TRH also reversed depression of ACh turnover [2] and HACU [13] produced by depressant drugs. Our earlier work also showed that the septohippocampal [10] and nucleus basalis-cortical cholinergic [7] pathways were involved in this response.

Most of the opiate research concerned with cholinergic mechanisms suggests that these drugs exert a cholinergic antirelease effect [3, 9, 14, 16], although several other investigators have shown that morphine increased central cholinergic activity [12,15]. The present study, as well as our earlier work with morphine, indicates that certain opiates produce arousal by activating central cholinergic mechanisms. However, it is unlikely that classical mu opiate receptors mediate this response since naltrexone not only was ineffective in blocking it, but enhanced the analeptic effect. Moreover, medial septal opiate receptors are known to decrease, rather than facilitate, cholinergic transmission of the septohippocampal pathway [4].

Our finding of an analeptic action of codeine may help to explain the recent report of its efficacy in the treatment of narcoleptic patients [5]. Although the authors assumed that codeine might be acting via endogenous opioid mechanisms, our investigations on opiate-induced arousal suggest no relationship between opioids and arousal mechanisms. Based on our present results, a more likely explanation would be that codeine exerts its antinarcoleptic effect by activating a cholinergic arousal system similar to that seen in our animals.

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