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Naloxone: Analeptic Action Unrelated to Opiate Receptor Antagonism?

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The effects on the duration of sleeping time (ST) of the opiate receptor antagonists, naloxone and naltrexone, were determined in rats anesthetized by intraperitoneal injection of ketamine, halothane, or pentobarbital. Intracerebroventricular administration of naloxone shortened the duration of sleeping time induced by all three anesthetic agents in a dose-related manner. Centrally administered naltrexone (240 μg) and systemically administered naloxone (50 mg/kg) prolonged the duration of pentobarbital sleeping time without altering duration of ketamine or halothane sleeping time. Naltrexone (120 μg) had no effect on the duration of ST. This study does not support a role for opiate receptor regulation of the duration of sleeping time. The evidence supports the hypothesis that naloxone may govern the duration of narcosis through the activation of an opposing arousal system in the CNS, unrelated to pharmacologic competition for opiate receptors. (Key words: Antagonists, narcotic: naloxone; naltrexone. Anesthetics, intravenous: ketamine; pentobarbital. Anesthetics, volatile: halothane. Polypeptides: endorphins; enkephalins. Receptors: opiate.)

THE PHYSIOLOGIC FUNCTION of the endogenous ligands and opiate receptors in the regulation of anesthesia remains controversial. Naloxone, a specific opiate antagonist, has been reported to antagonize the depth and duration of narcosis, analgesia, and cardiorespiratory effects of non-opiate anesthetic agents.[1-5] This implies that some of the effects of anesthetic agents may be mediated by opiate receptor mechanisms. Alternatively, naloxone antagonism could result from a nonspecific analeptic action or from activation of an opposing system independent of narcotic receptor sites.

In view of the controversial role of the endogenous ligands in anesthesia, a study was designed to investigate the possible role of opiate receptors in the modification of narcosis in laboratory animals. The effects of centrally administered naloxone and naltrexone on the duration of sleeping time (ST) have been determined in rats anesthetized with ketamine, halothane, or pentobarbital.

Methods and Materials

Male Sprague-Dawley rats (King Animal Laboratories, Oregon, Wisconsin) weighing 150–200 g, were housed under constant environmental conditions, and allowed access to food and water *ad libitum*. In the morning, between 9 A.M. and 11 A.M. each animal was weighed and given an intraperitoneal injection (ip) of either ketamine hydrochloride (175 mg/kg), halothane (1 ml/kg), or sodium pentobarbital (50 mg/kg). Immediately after the loss of the righting reflex (LRR), groups of rats received an ip injection of either naloxone (50 mg/kg) or 0.9 per cent saline. Other groups of anesthetized rats received an intracerebroventricular (ICV) injection of 120 or 240 μg of naloxone or naltrexone according to a method described previously.[6] Control ST were determined in rats receiving 0.9 per cent saline ICV. In preliminary studies in ketamine-anesthetized rats, we established a dose range for naloxone.[7] Equivalent molar doses of naltrexone were tested ICV. A total volume of 15 microliters of the test drug or 0.9 per cent saline was injected into the right lateral ventricle of the brain. The hydrochloride salt forms of the antagonists were dissolved in 0.9 per cent saline for injection. There were 10 to 18 rats per treatment group.

Awakening was defined as regaining the righting reflex (RRR). Sleeping time (ST), defined as the period between LRR and RRR, was recorded for all experimental groups. All rats given intraventricular injections were decapitated at the time of the RRR and the needle tract examined. Rats which did not show a definitive needle tract or had evidence of intracranial hemorrhage were excluded from the study.

Data are expressed as the mean ± SEM. Duncan's Multiple Range Test was used to compare and analyze data, and $P < 0.05$ was considered significant.

Results

There was no statistically significant difference between mean sleeping times of ip and ICV saline treated rats. The data were pooled for comparisons. Systemically administered naloxone, at the single dose tested, did not alter the ST induced by halothane and ketamine. However, pentobarbital-induced ST was increased by 28 per cent (table 1).

Intracerebroventricular administration of naloxone shortened the ST duration of rats anesthetized with ketamine, halothane, and pentobarbital. At the highest dose of naloxone tested, ST was shortened by 40 per cent for pentobarbital, 50 per cent for ketamine, and 47 per cent for halothane (table 1). In contrast, similar doses (240

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TABLE 1. Effect of Naloxone and Naltrexone on the Duration of Sleeping Time (ST) in Rats Anesthetized with Pentobarbital, Ketamine, and Halothane

Treatment Group	Mean Sleeping Time (min ± SEM)		
	Pentobarbital	Ketamine	Halothane
Saline	147.2 ± 7.8	95.0 ± 4.6	63.2 ± 2.6
Naloxone (ICV)			
120 μg (0.33 μM)	108.2 ± 8.0*	71.7 ± 2.5*	48.1 ± 4.1*
240 μg (0.66 μM)	88.9 ± 2.9†	47.7 ± 4.1†	29.4 ± 3.2†
Naltrexone (ICV)			
120 μg (0.32 μM)	141.8 ± 14.4	105.1 ± 4.5	61.7 ± 6.1
240 μg (0.64 μM)	175.9 ± 7.5*	89.1 ± 5.0	62.1 ± 6.3
Naloxone (ip)			
50 mg/kg	188.3 ± 6.9*	107.2 ± 5.6	57.8 ± 2.5

* Significantly different from control at 0.05 level (Duncan's Multiple Range Test).

† Significantly different from control and other treatment groups at 0.05 level (Duncan's Multiple Range Test).

μg) of the more potent narcotic antagonist, naltrexone, when centrally administered in anesthetized rats, significantly prolonged the duration of pentobarbital-induced ST by 20 per cent. This dose of naltrexone had no effect on the duration of ketamine- or halothane-induced ST. The lower dose of naltrexone (120 μg) had no effect on duration of ST induced by these agents.

Discussion

The present studies clearly demonstrate that centrally administered naloxone, but not naltrexone, shortens ST induced by ketamine, halothane, and pentobarbital. The results suggest that the analeptic effect of naloxone is not mediated by an opiate receptor mechanism. The fact that equivalent molar doses of naltrexone (0.32 μM and 0.64 μM naltrexone, and 0.33 μM and 0.66 μM naloxone) had no antianesthetic effect makes it highly unlikely that naloxone's activity is related to an opiate receptor mechanism. The doses of naloxone or naltrexone tested centrally or peripherally, were far above that ordinarily needed to antagonize narcotic drugs. The findings that naloxone antagonized narcosis induced by various CNS depressants in a dose-related manner is further evidence that sites, other than opiate receptors, may be responsible for the effect.

Hays *et al.*[8] have stressed that the antagonism of a response by the opiate antagonist naloxone is a necessary but insufficient criterion to implicate endogenous opiate mediation. More rigorous evidence, as emphasized by Sawynok *et al.*,[5] is needed to interrelate the mechanism of anesthesia and the opiate receptors. For instance, other opiate antagonists should provide similar effects as naloxone. Thus, naltrexone was ineffective in this respect. Naltrexone (N-cyclopropylmethyl congener of naloxone) is a potent narcotic antagonist with undetectable or minimal agonist activity, and a longer duration of action than naloxone. Animal studies indicated its relative opiate antagonist potency was 39 times that of nalorphine and two to three times that of Naloxone.[9] Naltrexone is four to five times as potent a binder to opiate receptor sites as naloxone.[10-12] In humans, it is 17 times as potent as naloxone in precipitating acute abstinence.[13]

In addition, agents which inhibit the breakdown of endogenous opioid peptides should potentiate a response.[5] Treatment with such agents failed to modify the duration of pentobarbital anesthesia in mice.[14] Recent studies have demonstrated that naloxone possesses other pharmacologic properties which are not related to competition for narcotic receptors. For example, naloxone has been observed to possess local anesthetic activity *in vivo* with a therapeutic index similar to lidocaine in addition to its analeptic action.‡ Other studies have demonstrated that naloxone may act as a GABA antagonist.[15] We have reported that narcosis induced by diazepam, effects of which may be mediated by GABA interactions,[16] is not antagonized by naloxone.[17]

We contend that analeptic agents do not arouse from narcosis by stimulating the respiratory, cardiovascular, or temperature centers, but by directly affecting physiologic arousal systems of the brain.[6] Horita and Carino[18] observed that a cholinergic mechanism may be responsible for the analeptic action of naloxone in rabbits anesthetized with pentobarbital. In other studies, we observed that atropine pretreatment of rats, anesthetized with ketamine,[7] pentobarbital, or halothane (unpublished observations) abolished the analeptic action of naloxone. The analeptic action of naloxone may result from the activation of an opposing physiologic arousal system which is mediated cholinergically.

In another behavioral model, differential effects of naloxone secondary to the route of administration have been reported.[19] Differences in anti-anesthetic action of other analeptic agents have been observed in amobarbital-induced narcosis in rats. This action was dependent upon the route of administration (unpublished observation). Although cerebral perfusion with naloxone in dogs antagonized the depressant circulatory effects of halothane,[4] the anti-anesthetic actions of naloxone have not been observed consistently after peripheral administration.[2,3,5,14,20-22] Based on the fact that naloxone has a relatively short duration of action in rat brains, Arndt and Freye speculated that brain naloxone concentrations did not accumulate in sufficient concentrations after pe-

‡ Kraynack, BJ, Cohn ML: Naloxone: A narcotic antagonist with local anesthetic properties (abstract). Annual Meeting, International Anesthesia Research Society, March 1978, p 14.

ripheral administration to antagonize the duration of anesthesia.

In summary, we have demonstrated the analeptic action of centrally administered naloxone, but not naltrexone. The evidence suggests that naloxone may govern the duration of narcosis through the activation of an opposing, cholinergic arousal system in the CNS. A nonspecific analeptic action of naloxone cannot be excluded. Regardless of the specific mechanism, it appears that the analeptic effect is not related to pharmacoligic competition for opiate receptors. The inference that anesthesia and narcotic drug action share a common mechanism must await more definitive lines of evidence to link the mechanism of narcosis with the opiate receptor system.

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