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Memory Retrieval Enhancement by Kappa Opioid Agonist and Mu, Delta Antagonists

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ILYUTCHENOK, R. Y. AND N. I. DUBROVINA. *Memory retrieval enhancement by kappa opioid agonist and mu, delta antagonists.* PHARMACOL BIOCHEM BEHAV 52(4) 683–687, 1995.—The present study sought to identify specific opioid receptor subtypes involved in the modulation of reactivation of amnesic or forgotten memory traces by use of a one-trial inhibitory avoidance training procedures in mice. The effects of naloxone, ICI 174,864 (mu and delta opioid receptor antagonists, respectively) and dynorphin (kappa agonist) were investigated. The results indicated that preretention test administration of naloxone (2 mg/kg) or ICI 174,864 (3 mg/kg) attenuated the amnesia and forgetting as indicated by prolongation of step-through latency. On the other hand, the activation of kappa opioid receptors by dynorphin (1 mg/kg) also showed reactivating effects both after amnesia and forgetting. On the basis of the parallelism of the effects for mu and delta opioid receptor antagonists and kappa agonist, and on the finding that all three opioids demonstrated a different degree of reactivation of amnesic and forgotten memory traces, it was concluded that mu, delta, and kappa opioid receptors contribute to the modulation of amnesia and forgetting by independent mechanisms.

Memory Passive avoidance Amnesia Forgetting Opioid receptors Naloxone ICI 174,864 Dynorphin

OPIOIDS produce their principal effects by binding to at least three different types of receptors, the mu, delta, and kappa opioid receptors (4,16,24). With the recent development of the number of ligands that are highly selective for specific opioid receptor types, it is now possible to study whether selective activation or antagonism of specific types of opioid receptors influences learning and memory. The functional role of the mu and delta receptors has been extensively studied, as compared with kappa agonists and antagonists. In general, the mu and delta agonists tend to interfere with learning and memory and to produce retrograde amnesia. Alternatively, the antagonists such as naloxone and ICI 174,864 tend to facilitate them, to increase retention of recently learned task and to alleviate amnesia, although there are many exception to this rule, depending on the experimental paradigms used and the particular variables manipulated (7,9,15,23,27).

Dynorphin is an endogenous opioid peptide that has been found to act on kappa receptors. The function of dynorphin and other kappa agonists in central nervous system has been found to include modulation of locomotor activity, reward, and memory (8,18,20,21,26). Studies of the effects of kappa selective agonists on memory have yielded highly conflicting results. Izquierdo et. al. (10) reported that posttraining administration of dynorphin did not affect retention on either active or passive avoidance tasks. Similarly, Tilson et. al. (28) found that intracerebroventricular injection of dynorphin had no effect on retention of a inhibitory avoidance task. In mice, dynorphin impaired retention of passive avoidance task, without altering retention in either a discrimination task or a habituation of exploratory activity (8). However, it is shown that the retention impairment produced by adrenalectomy is attenuated by kappa selective agonists (12). Thus, although there is clear evidence that kappa agonists can influence memory, the effects are unlike those found with other opioid peptides. As yet, no evidence suggests a role for the activation of kappa receptors in memory retrieval.

One interpretation of the literature concerning the neurobiological effects of mu, delta, or kappa agonists and presumably the opioid receptors that they activate, is that these have different, if not opposite, activities.

The purpose of the present experiments was to examine in more detail the possible differential effects of mu and delta antagonists and kappa agonist on reactivation of amnesic and forgotten memory traces as well as to determine the role of

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multiple opioid receptor types in mediating the amnesia from detention and forgetting.

METHOD

Animals

Male mice of the BALB/c strain were used. They were housed in an environmentally controlled animal room. Animal rooms were on a 12L : 12D cycle with lights going on at 08:00 h. Food and water were available at all time. Twenty-four hours before the start of an experiment, the mice were weighed and the median body weight was 20 g, with a range of 17-22 g. Mice were assigned randomly to groups of 13-18. Testing occured between 11:00 and 16:00 h.

Procedure

The step-through inhibitory avoidance apparatus used was similar to that previously described (11). On the training trial, the mice placed in a start compartment facing away from the door and allowed to enter the shock compartment. When the animal entered the shock box with all four feet, the step-through latency was recorded, the entry was closed with a sliding door and a foot shock (1 mA, 2.0 s) was delivered. The mouse was then immediately returned to its home cage. On the retention test 1-8 days later, the mouse was placed in a start compartment as in the training session and the step through latency (maximum of 180 s) was recorded. High latencies are interpreted as reflecting good retention, and low latencies are taken to indicate poor retention. There was one pre-training trial, during which the animals were habituated to the experimental conditions.

"Psychogenic" amnesia was elicited by 5 min detention of the animal in the shock compartment immediately after foot shock on the training trial (25).

Forgetting was produced by a 21 days training to testing interval.

In the first set of experiments, animals were injected IP 30 min before the retention test on day 2 with one of the drugs listed below:

(A) physiological saline, $n = 17$
(B) naloxone 2 mg/kg, $n = 13$
(C) ICI 174,864 3 mg/kg, $n = 18$
(D) dynorphin 1 mg/kg. $n = 18$

All conditions drugs administration were identical for the second set of experiments, except that drugs were injected on day 22: (a) $n = 15$; (b) $n = 15$; (c) $n = 15$; (d) $n = 13$.

Drugs

Naloxone-hydrochloride (Serva, Germany), ICI 174,864 (diallyl-tyr-aib-aib-phe-leu) obtained from Imperic Chemical Industries (England), dynorphin A (1-13; Vector, Russia) were dissolved in sterile physiological saline just before injection. Drugs or saline vehicle was injected in a volume of 10 ml/kg. The results were evaluated statistically by a two-way analysis of variance (ANOVA). Post hoc comparisons were assessed with the use of Sheffe method. In every case, the minimum acceptable level for statistical significance was $p < 0.05$.

RESULTS

To determine if blockade of the mu or delta opioid receptors and activation of the kappa receptors had any effect on learning, mice were administered naloxone (2 mg/kg), ICI 174,864 (3 mg/kg) or dynorphin (1 mg/kg) 30 min before training. These results are summarized in Table 1. The groups injected with saline vehicle (control group) or drug failed to demonstrate any consistent or significant change in step through latencies. Furthermore, high latencies in this groups indicated a good passive avoidance acquisition and can serve as controls for performance effects and the degree of success of the amnesic treatment.

In the first experiment, the effects of naloxone, ICI 174,864 and dynorphin on passive avoidance retrieval were tested. As seen in Fig. 1, amnesia induced by the detention of an animal in the shock compartment is appearant at all training-retention-test intervals.

An overall 4 (drug) × 8 (day) two-way ANOVA analysis of variance indicated a significant drug effect [$F(3, 62) = 22.87$, $p < 0.0001$], significant day difference [$F(7, 434) = 18.53$, $p < 0.0001$], and a significant (drug × day) interaction [$F(21, 434) = 6.16$, $p < 0.0001$]. Scheffe's posthoc tests revealed that the pretesting injection of naloxone, ICI 174,864, or dynorphin significantly increased step through latencies relative to saline-treated control ($p < 0.05$).

The ability to maintain improvement of memory retrieval induced by naloxone was 6 days. In addition, naloxone produced enhancement of its effect from 30 min to day 4 and only then did a sharp decrease of effect registered.

Animals receiving ICI 174,864 and dynorphin before testing showed a significant facilitation of memory retrieval from 30-min to 2-day postinjection ($p < 0.05$). Latency values returned to baseline level of control group after 3-day test period following drugs administration.

The purpose of the second set of experiments was to deter-

TABLE 1

EFFECTS OF ACTIVATION OF THE KAPPA RECEPTORS AND BLOCKADE OF DELTA OR MU RECEPTORS ON LEARNING

	Mean Step Through Latency (M ± SEM)					
		After Training, Day				
Treatment Group	Before Training	1	2	3	4	5
Saline ($n = 15$)	15 ± 1	140 ± 19	137 ± 19	129 ± 17	102 ± 19	94 ± 15
Naloxone ($n = 14$)	15 ± 1	128 ± 18	127 ± 18	130 ± 19	99 ± 21	93 ± 19
ICI174,864 ($n = 15$)	16 ± 2	138 ± 18	127 ± 17	114 ± 18	89 ± 18	95 ± 16
Dynorphin ($n = 14$)	14 ± 2	140 ± 18	131 ± 18	121 ± 14	100 ± 21	94 ± 17

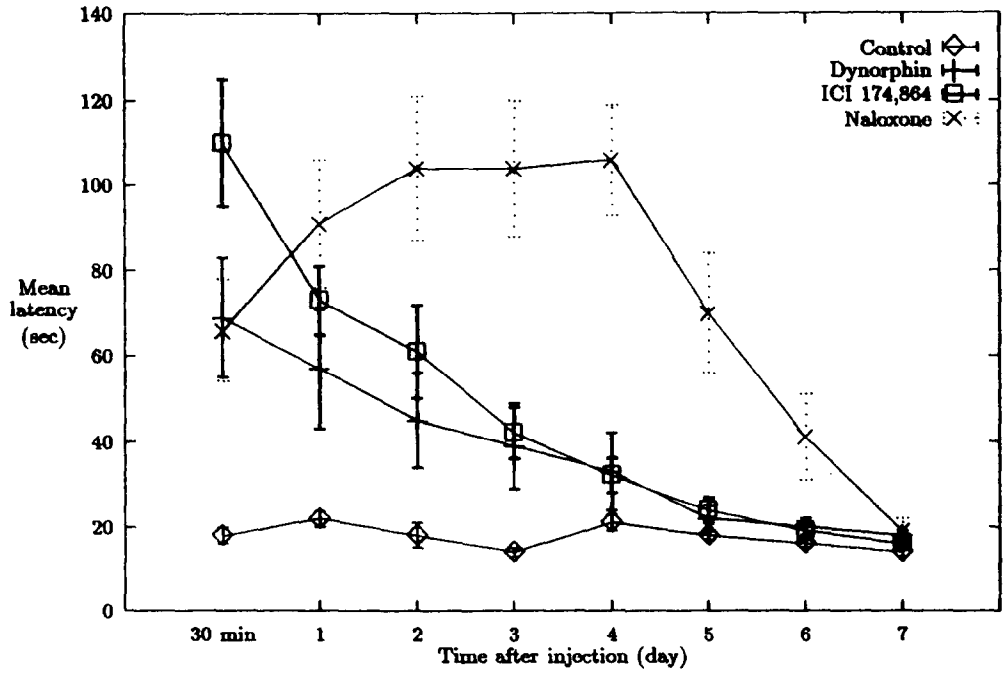

FIG. 1. Reactivation of passive avoidance retrieval by naloxone (2 mg/kg), ICI 174,864 (3 mg/kg), and dynorphin (1 mg/kg) administered 30 min prior to testing on second day after training and amnesic treatment.

mine if naloxone, ICI 174,864, and dynorphin show similar facilitating effects on memory retrieval processes impaired by a prolonged interval between acquisition of information and its retrieval. After a 3-week training to test interval, there was a retention deficit in mice. This forgetting was spontaneous, in that it occurred without any interference manipulation and poor retention from control animals was registered (Fig. 2).

As expected, blockade of the mu or delta receptors and activation of the kappa receptor increased the step-through latencies in the avoidance apparatus (Fig. 2). The results of overall ANOVA 4(drug) × 6(day) indicated that they were a significant drug [$F(3, 54) = 15.63, p < 0.0001$ and day [$F(5, 270) = 23.85, p < 0.0001$] differences and (drug × day) interaction [$F(15, 270) = 4.50, p < 0.0001$]. Posthoc comparisons showed that all three agents alleviated forgetting relative to saline control ($p < 0.05$).

An attempt was made to determine the duration of drug effects. Dynorphin produced significant improvement of memory retrieval compared to saline control only 1 day after drug injection ($p < 0.05$).

The effect of pretesting administration of naloxone is retained for 3-day postinjection ($p < 0.05$). The improvement of memory retrieval induced by ICI 174,864 was obtained for 4-day ($p < 0.05$) and at 5-day postinjection the step through latency values approached baseline level.

Visual examination of the individual groups with ICI 174,864 or dynorphin (compare Figs. 1 and 2) indicated that the level of avoidance performance in these animals appears not to be different between the amnesia and forgetting conditions. A 2(group) × 6(day) ANOVA comparing the ICI 174,864 group from the "psychogenic" amnesia experiment with the corresponding group from the spontaneous forgetting experiment failed to reveal significant differences between groups [$F(1, 31) = 0.56, p > 0.5$] and an interaction of group and day [$F(5, 155) = 1.47, p > 0.2$]. The dynorphin groups also showed no significant differences between the two experimental paradigms ($p > 0.05$). The only clear difference considering comparable experimental conditions is revealed by naloxone after "psychogenic" amnesia and spontaneous forgetting, respectively. A 2(group) × 6(day) ANOVA analysis revealed a significant group difference [$F(1, 26) = 9.26, p < 0.0006$] and day difference [$F(5, 130) = 3.85, p < 0.004$], and group × time interaction [$F(5, 130) = 7.63, p < 0.0001$.

DISCUSSION

The present study shows that the three opioid ligands (naloxone, ICI 174,864, and dynorphin) all improved memory retrieval in two experimental situations. The results of these experiments provide a basis for the separation of biological functions associated with mu, delta on the one hand and kappa opioid receptors on the other hand. Indirect evidence in support of this conclusion includes results of behavioral changes of very different nature, induced by mu, delta, and kappa agonists and antagonists. Previous studies have demonstrated that opioids may have reinforcing or aversive effects depending on the receptor type with which they interact. Thus, mu and delta receptor agonists function as positive reinforcers in several experimental paradigms whereas selective kappa agonists produce aversive states (1,20). The similar antagonism of activation of mu, delta, and kappa receptors was observed in locomotor activity, conflict and operant behaviors, in the conditioned place preference paradigm, and in intracranial self-stimulation (2,6,13,21,26,28). The opposite reaction also occurred on membrane level: mu and delta selective agonists opened the special type of potassium channel while kappa agonists controlled calcium permeability (3).

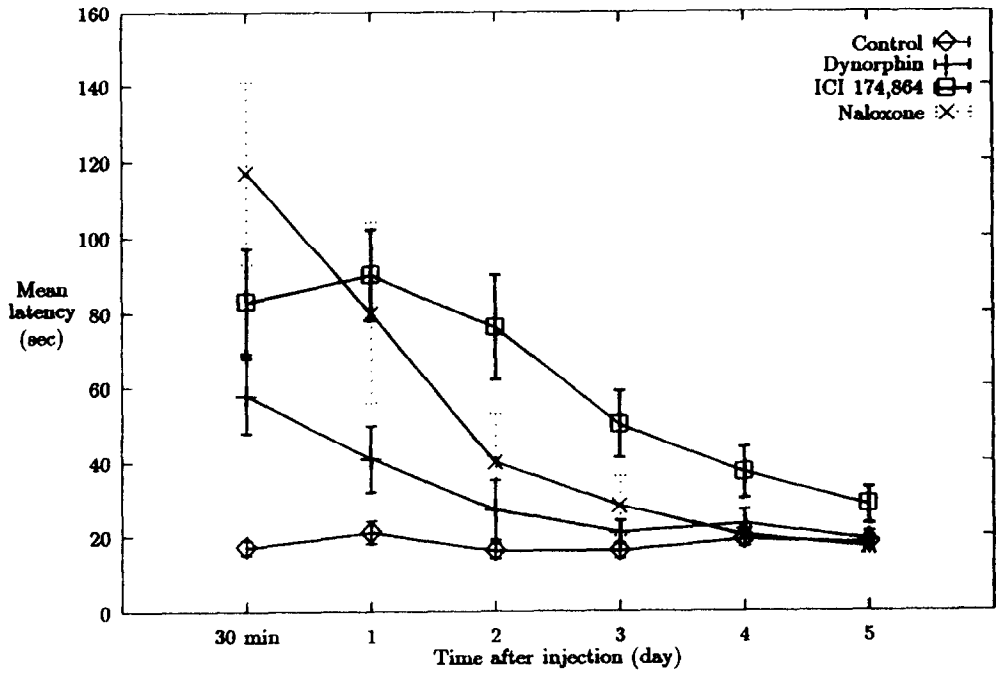

FIG. 2. Alleviation of spontaneous forgetting in the groups treated with naloxone (2 mg/kg), ICI 174,864 (3 mg/kg), or dynorphin (1 mg/kg) 30 min before retention test, 22 days after the training.

The effects of opioid peptides on learning and memory are much less clear and more conflicting (7,22). Kappa agonists (dynorphin, bremazocine, U50,488) are known to impair acquisition in some experimental paradigms, like mu and delta agonists (8,18,26). One-trial passive avoidance and its mediation by opioid peptides has been studied with more consistent results. It is shown that physiological effects of mu and delta agonists were clearly different than those of kappa agonists. For example, posttrial administration of DADLE significantly impaired retention of a step through passive avoidance task, whereas injection of dynorphin had no effect (28). Possible functional role of mu, delta, and kappa receptors in amnesia and forgetting is still unclear, although some studies have suggested that unlike mu and delta agonists, dynorphin does not cause retrograde amnesia (10,14,23). Although several investigators have shown that posttraining administrations of dynorphin did not affect retention of passive avoidance tasks (18), the present results showed an effectiveness of this drug in reestablishment of amnesic and forgotten memory traces.

The data reported here suggest that the effects of naloxone, ICI 174,864, and dynorphin on retrieval processes are due to direct influences on memory and are not indirectly mediated through influences on performance variables such as locomotor activity or foot shock reactivity. First, it is clear from our findings that the memory-modulating effects of opioids are more long-lasting than those obtained with other behavioral tasks. For example, the memory-enhancing action of posttraining administration of naloxone lasts for 6 days after injection. Further, opioid effects on memory are not restricted to one-trial inhibitory avoidance tasks in which response latency is used as the measure of retrieval. The learning and memory enhancement or impairment have been found with several types of training tasks, including active avoidance, aversively motivated discrimination, habituation, latent inhibition and appetitively motivated spatial learning [see (15,17, 19,22) for review]. Finally, it is important to note that our results, which are based on analysis of opioid effectiveness in the reactivation of amnesic and forgotten memory traces, are only partially consistent with those on motor function and behavioral reactivity.

Although the results of these experiments leave open many possible interpretations concerning the mechanisms involved in the facilitation of retrieval processes, they are certainly highly consistent with hypothesis that mu, delta, and kappa receptors mediate independent remembering following "psychogenic" amnesia and spontaneous forgetting. Our results showing that the blockade of mu and delta and the activation of kappa receptors improve retrieval of inhibitory avoidance, impaired by amnesic agent or forgetting, are interesting in light of a recent study by Dubrovina and Ilyutchenok (5). Subsequent behavioral experiments revealed that the activation of dopaminergic system (chiefly D_2 dopamine receptors) determines the improvement of retrieval of passive avoidance response in the paradigm of "psychogenic" amnesia. On other hand, it is shown the importance of blockade of various components of benzodiazepine/GABA-ionophore receptor complex in facilitating memory retrieval following spontaneous forgetting. These results, in combination with those of the present study, suggest that retrieval deficits after amnesia and forgetting may be related to a dysfunction of interaction between GABA, dopamine, and opioid systems.

It is intriguing that the blockade of mu receptor appears to play upon dopaminergic and GABAergic systems that have independently been implicated in memory processes after amnesia and forgetting. The facilitating effects of naloxone administration on the retrieval of amnesic memory trace could be due to a release of the dopaminergic system from opioid-caused inhibition. A wide variety of information indicates that opiates and opioid peptides are capable of inhibiting dopamine activity through opioid receptor mechanisms, probably

of the mu receptor subtype, in both the soma/dendritic and the terminal fields of dopaminergic neurons (29). It is probably that regulation of memory recovery by naloxone after spontaneous forgetting dependent upon the interaction of the mu receptors with components of the benzodiazepine/GABA-chloride ionophore receptor complex. Additional studies are needed to clarify the role that these three opioid receptor populations play in the interaction with "classical" neurotransmitter systems in the amnesia and forgetting.

In summary, the current study provides strong evidence of contribution of mu, delta, and kappa receptors in reactivation of memory impaired by amnesic agent (detention) or spontaneous forgetting.

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