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PD134308, a selective antagonist of cholecystokinin type B receptor, enhances the analgesic effect of morphine and synergistically interacts with intrathecal galanin to depress spinal nociceptive reflexes

(neuropeptide/spinal cord/hot plate test/pain)

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ABSTRACT **The effects of systemic PD134308 [0.1–3 mg/kg; an antagonist of the cholecystokinin (CCK) type B receptor], morphine, and intrathecal (i.t.) galanin (GAL) on the excitability of the spinal nociceptive flexor reflex and in the hot plate test were examined in rats. PD134308 caused a weak naloxone-reversible depression of the flexor reflex and a moderate antinociceptive effect in the hot plate test. However, PD134308 significantly potentiated the antinociceptive effect of morphine as well as its depressive effect on the flexor reflex. PD134308 and i.t. GAL synergistically depressed the flexor reflex, an effect that was reversed by naloxone. Finally, the magnitude and duration of the depression of the flexor reflex by morphine were synergistically increased by coadministering PD134308 and GAL i.t. The results demonstrated that a CCK antagonist directed to the central CCK type B receptor potentiates the analgesic effects of opioids and nonopioid drugs at the spinal level, thus supporting the notion that CCK in the central nervous system may be an endogenous, physiological opioid antagonist.**

Cholecystokinin (CCK) (1) is present in many areas of the central nervous system (CNS), primarily as the sulfated C-terminal fragment CCK-8 (2–4). This peptide fulfills many of the criteria for a neurotransmitter and may have a role in various CNS functions (see ref. 5). In the spinal cord genuine CCK-like immunoreactivity has been described in local neurons in the dorsal horn and in neurons in lamina X (6–9), areas of the spinal cord which are believed to have a role in nociception.

Studies on the effects of exogenously applied CCK and related peptides on pain modulation in rodents have given different results (see ref. 10). On the one hand, after various routes of administration CCK has been found to cause analgesia, although the effect has not been consistent across species, doses, and various tests of nociception (11, 12). On the other hand, CCK has been found to reduce the analgesic effect of the morphine and β-endorphin (13–16).

In electrophysiological studies most reports have indicated that CCK has an excitatory effect on spinal neurons (17, 18). We have shown that intrathecal (i.t.) administration of the sulfated form of CCK facilitated the spinal nociceptive flexor reflex over a wide dose range (0.01–1 μg), suggesting lack of analgesic effect of CCK at the spinal level, and we found that CCK administered prior to i.t. morphine could prevent the full development of morphine-induced reflex depression and substantially enhance the facilitatory effect of morphine (19). These results were supported by single cell recordings in the rat dorsal horn (20).

Evidence has been presented that there may be multiple receptors for CCK (21) and that the most abundant type of receptor for CCK in the periphery differs from that in the CNS (21, 22). The peripheral type receptor has been designated as CCK-A and the central one as CCK-B (22). More recently a variety of CCK antagonists have become available (see ref. 23), opening up possibilities to resolve the role of CCK in pain systems. Thus the weak, nonselective CCK receptor antagonists proglumide and benzotript potentiate the antinociceptive effect of morphine and other opioids (24–26). Studies with more recently developed CCK-A antagonists (Devazepide, formerly MK 329; L-365,031) and CCK-B antagonists (L-365,260) further support a role for CCK as a physiological opioid antagonist, since these drugs enhanced the analgesic effect of morphine (16, 27, 28). Furthermore, these three antagonists abolish tolerance to chronically administered morphine. The rank order of potency of these antagonists for enhancing morphine analgesia was L-365,260 > MK-329 > L-365,031, which correlated with their potency to block the central CCK-B receptor in rats, indicating that the site of CCK/opioid interaction is in the CNS and the interaction is mediated by CCK-B receptors (28).

Another highly selective and potent CCK-B receptor antagonist, PD134308, has recently been developed (29). It has been shown to block the CCK-induced increase in the frequency of spontaneous action potentials in the ventromedial nucleus of the hypothalamus, an effect known to be mediated by the CCK-B receptor, and was found to be a highly effective anxiolytic agent (29). We have now examined the effect of PD134308 on nociception by using two experimental models, the spinal nociceptive flexor reflex in decerebrate, spinalized, unanesthetized rats and a nonreflexive behavioral test of nociception, the hot plate test. It was shown that PD134308 potentiates the analgesic effect of morphine and of morphine combined with galanin (GAL). GAL is a neuropeptide (30) that may have an endogenous antinociceptive effect by antagonizing the facilitatory effect of substance P and calcitonin gene-related peptide (31) and potentiating the spinal analgesic effect of morphine (32).

MATERIALS AND METHODS

Physiological studies were carried out on Sprague–Dawley rats of both sexes (250–300 g) as described previously (19).

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Abbreviations: CCK, cholecystokinin; CNS, central nervous system; i.p., intraperitoneally; i.v., intravenously; i.t., intrathecally; GAL, galanin; ANOVA, analysis of variance.

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Table 1. Magnitude and duration of the depressive effect of PD134308, morphine, and GAL and the combination of these drugs on the flexor reflex

Drugs	*n*	Reflex depression	
		Magnitude, %	Duration, min
PD134308 (1 mg/kg i.v.)	6	24.5 ± 5.9	28.6 ± 7.6
Morphine (1 mg/kg i.v.)	8	38.1 ± 10.7	60.5 ± 27.2
PD134308 (1 mg/kg i.v.) + morphine (1 mg/kg i.v.)	6	62.5 ± 11.2[a,c]	195.3 ± 24.2[a,c]
GAL (100 ng i.t.)	15	0	0
GAL (100 ng i.t.) + morphine (1 μg i.t.)	5	58.5 ± 15.1[b]	80.1 ± 15.2
GAL (100 ng i.t.) + PD134308 (1 mg/kg i.v.)	4	66.0 ± 15.9[a,c]	152.2 ± 38.9[b,c]
GAL (100 ng i.t.) + morphine (1 mg/kg i.v.) + PD134308 (1 mg/kg i.v.)	4	85.6 ± 13.7[d,e]	250.2 ± 14.1[d,f,g]

Data were analyzed by two-way ANOVA followed by Scheffe *F* test: a = compared with morphine, $P < 0.01$; b = compared with morphine, $P < 0.05$; c = compared with PD134308, $P < 0.01$; d = compared with morphine + PD134308, $P < 0.05$; e = compared with morphine + GAL, $P < 0.05$; f = compared with morphine + GAL, $P < 0.01$; and g = compared with PD134308 + GAL, $P < 0.01$.

Briefly, the magnitude of the hamstring flexor reflex in response to activation of high threshold afferents was examined in decerebrate, spinalized (spinal-cord-transected), unanesthetized rats by recording the electromyogram from the posterior biceps femoris/semitendinosus muscles. Anesthetized rats were mounted in a stereotaxic frame, decerebrated, and spinalized at segments Th8–9. In some rats, an i.t. catheter (polyethylene, outside diameter 0.61 mm) was implanted caudal to the transection with its tip at the lumbar enlargement (segments L4–5). The reflex was continuously elicited by test stimuli of single electric shocks (1/min) to the sural nerve (1 ms, 5 mA) or subcutaneous shocks in the sural nerve innervation area (1 ms, 10 mA) to activate C-fibers (33). The number of potentials elicited during the reflex was integrated over 2 s. A stable baseline reflex magnitude was established for at least 20 min prior to the injection of drugs.

Behavioral tests were performed on male rats. The antinociceptive effect of the drugs was assessed with a hot plate (IITC, Woodland Hills, CA) at 54 ± 0.2°C. The latency to licking a hindpaw was determined to an accuracy of 0.1 s. Before any drugs were administered, the rats were habituated by being tested on the hot plate for 5 days to obtain a stable control response value. The average baseline response latency was 4.2 ± 0.2 s on the day before drug administration, and there was no significant difference between groups.

With regard to drugs, morphine hydrochloride (Apoteksbolaget, Stockholm), PD134308 (Parke-Davis, Cambridge, U.K.; see ref. 29 for structure), and naloxone (DuPont) were injected in a volume of 0.2 ml i.v. in the physiological studies and i.p. in the behavioral experiments. Porcine GAL (Bachem) and sulfated CCK (26–33) (Cambridge Research Biochemicals, Cambridge, U.K.) were dissolved in 0.9% saline and frozen in aliquots at −70°C. GAL and CCK were injected i.t. in a 10-μl volume followed by 10 μl of saline to flush the catheter.

For statistical evaluation, the effects of drugs on the magnitude of the flexor reflex were expressed as percent change from baseline, which was defined as 100%. The analgesic effects of drugs on the hot plate test were expressed as percent increase in reaction latency to control value, set as 100% and obtained prior to drug administration. The postdrug effect (DL) was calculated as the percent change in reaction time from baseline latency (BL) values by using the formula $[(DL - BL)/BL] \times 100\%$. Statistics were performed with one- and two-way analysis of variance (ANOVA), which was followed by the Scheffe *F* test and Dunnett's test, respectively. All data are presented as mean ± SEM.

RESULTS

Potentiation of the Analgesic Effect of Morphine by PD-134308. PD134308 (1 mg/kg) injected i.v. caused a weak depression of the flexor reflex by about 25% below control reflex magnitude for about 30 min (Table 1, Fig. 1). This effect was readily reversed by i.v. naloxone (1 mg/kg, Fig. 1). PD-134308 also antagonized the facilitation of the flexor reflex by 100 ng and 1 μg i.t. CCK (Fig. 2). PD134308 (1 mg/kg) and morphine (1 mg/kg) interacted synergistically to depress the flexor reflex (Table 1, Fig. 3), and after coadministration the reflex was depressed about twice as much as in response to each drug alone, and the duration of the depression was about 3 times as long as after morphine alone (Table 1).

PD134308 at low doses (0.1 or 0.3 mg/kg) had no effect on lick latency in the hot plate test, whereas larger doses had a weak, but significant, analgesic effect for 20 min (1 mg/kg) and 40 min (3 mg/kg) (Fig. 4). Morphine at 1 mg/kg caused moderate analgesia for about 50 min, and PD134308 in a dose-dependent fashion potentiated the analgesic effect of morphine (Fig. 5). With the three higher doses of PD134308 the analgesic effect of morphine was significantly potentiated at all intervals examined, starting 10 min after injection. The duration of the analgesia was increased from 50 to 90 min with 0.3 mg/kg and to 130 min with PD134308 at 1 or 3 mg/kg.

Interaction of i.t. GAL with Systemic PD134308 and Morphine. One hundred nanograms of GAL briefly facilitated the flexor reflex without causing reflex depression, and 1 μg of

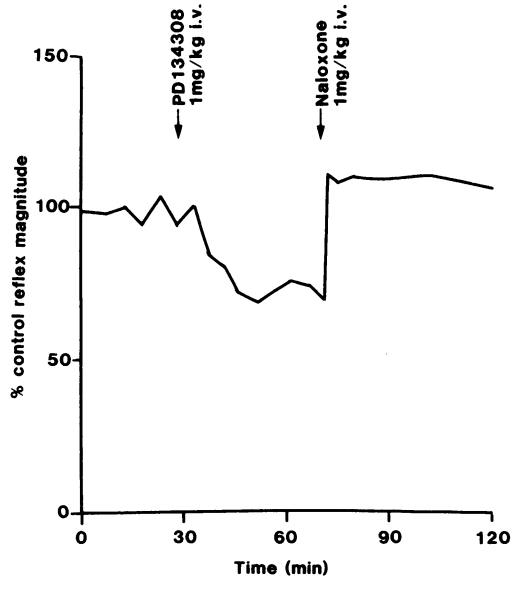

FIG. 1. Effect of PD134308 (1 mg/kg i.v.) on spinal reflex excitability. Control reflex magnitude in this and subsequent figures is defined as 100%. PD134308 caused moderate reflex depression starting about 5 min after administration, which was reversed by naloxone.

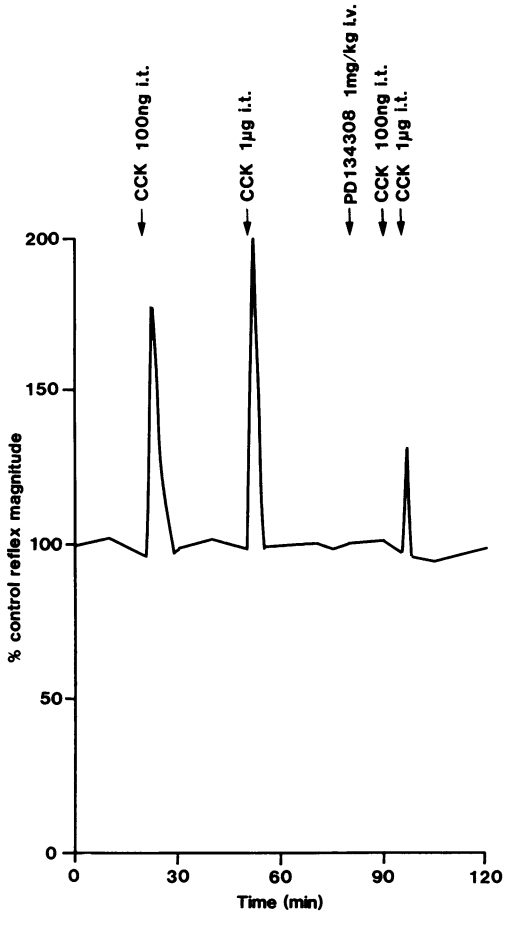

FIG. 2. CCK (100 ng and 1 μg) administered i.t. briefly facilitated the flexor reflex. PD134308 administered 10 min prior to i.t. CCK totally antagonized the effect of 100 ng of CCK and significantly reduced facilitation by 1 μg of CCK.

morphine caused a weak facilitation followed by reflex depression for about 1 hr. When GAL and morphine were coadministered i.t. at these doses the reflex depression

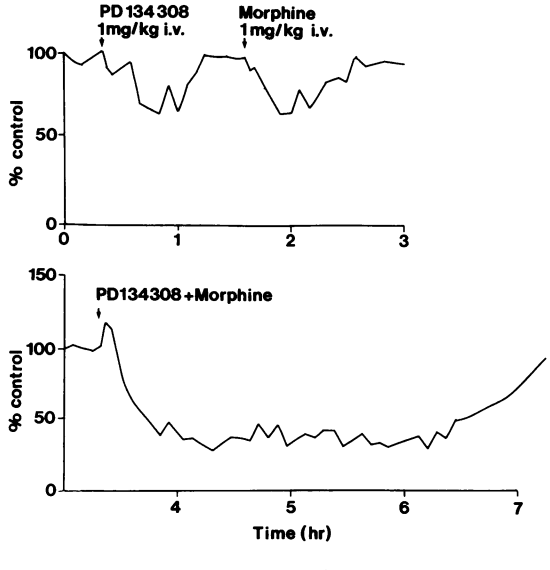

FIG. 3. (*Upper*) PD134308 (1 mg/kg) or morphine (1 mg/kg) moderately depressed the flexor reflex for 30–60 min. (*Lower*; continuation of abscissa from *Upper*) The magnitude and duration of depression were greatly enhanced by coadministration of the two drugs.

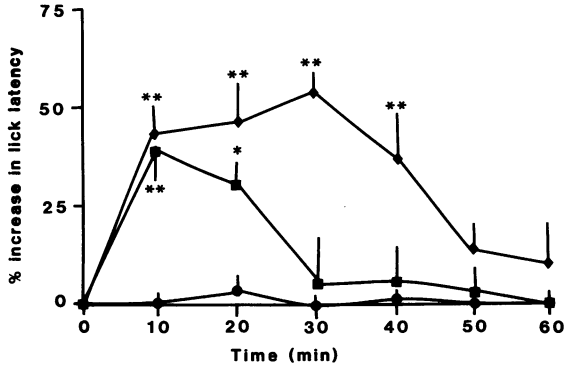

FIG. 4. Effect of PD134308 at 0.3 (●), 1 (■), or 3 (◆) mg/kg i.p. on the hot plate test. The two highest doses had a moderate antinociceptive effect, while no effect was observed with 0.1 mg/kg (not shown) or 0.3 mg/kg. $n = 5$ in each group. One-way ANOVA was followed by Dunnett's test: * = $P < 0.05$ and ** = $P < 0.01$.

became significantly stronger, and its analgesic duration was also increased (Table 1, Fig. 6).

The effects of GAL at 100 ng i.t. and PD134308 at 1 mg/kg i.v. administered within a 5-min interval, are summarized in Table 1 and illustrated in Fig. 6. Both the magnitude and duration of reflex depression by PD134308 at 1 mg/kg i.v. were significantly potentiated by GAL at 100 ng i.t. In fact, the reflex depression by PD134308 plus GAL was significantly stronger and longer lasting than after morphine at 1 mg/kg. Furthermore, the depressive effect of GAL plus PD134308 was reversed by naloxone.

An extremely strong potentiation of the effect of i.v. PD134308 plus i.v. morphine was observed after i.t. administration of GAL (Table 1, Fig. 7). The reflex was depressed by about 85% below control levels, which was significantly greater than the effect of GAL plus morphine or PD134308 plus morphine. The duration of the reflex depression was about 250 min, which was significantly longer than the effect of GAL plus morphine, PD134308 plus morphine, or PD134308 plus GAL.

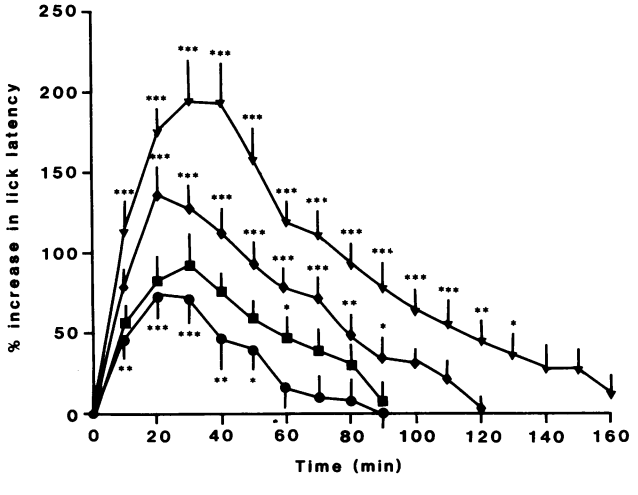

FIG. 5. Effect of 1 mg/kg morphine by itself (●) and combined with PD134308 at 0.1 (■), 0.3 (◆), or 1 (▼) mg/kg on the hot plate test. PD134308 at 3 mg/kg plus morphine was similar to PD134308 at 1 mg/kg (not shown). The effect of morphine alone was compared with predrug response latency with one-way ANOVA. The effects of morphine plus various doses of PD134308 were compared with the effect of morphine alone with 2-way ANOVA followed by Dunnett's test. * = $P < 0.05$; ** = $P < 0.01$; and *** = $P < 0.001$. $n = 10$ in each group.

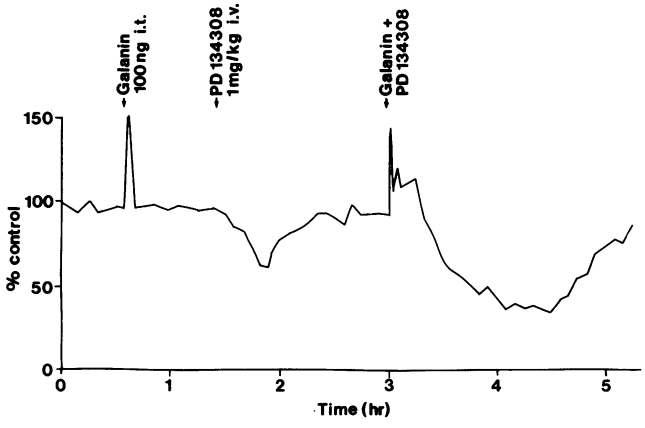

FIG. 6. Effects of GAL at 100 ng i.t., PD134308 at 1 mg/kg i.v., and the combination of the two drugs on the excitability of the flexor reflex.

DISCUSSION

In the present work we have shown that systemic PD134308, a specific CCK-B antagonist (29), dose-dependently potentiates the analgesic effect of morphine both in the hot plate test and in the flexor reflex model in spinalized rats, supporting the hypothesis that CCK-8 is an endogenous opioid antagonist (14, 15). Since PD134308 has only low affinity to the CCK-A receptor (29), the results strongly indicate that the interaction between CCK and opioids occurs through CCK-B receptors in the CNS (28). The potentiating effect of PD134308 on morphine-induced analgesia is robust, apparently stronger than the effect reported with another CCK-B antagonist, L-365,260 (28). Also, PD134308 tested over a 30-fold dose range did not show a bell-shaped distribution of dose effectiveness, which was seen with L-365,260 (28). It is, however, difficult to make a direct comparison between the efficacy of these two drugs in potentiating the analgesic effect of morphine, since different animal models were employed.

Systemic PD134308 by itself at high doses had a weak analgesic effect and depressed the flexor reflex, and these effects were naloxone sensitive. It seems unlikely that this effect was due to a direct interaction of PD134308 with an opioid receptor, since PD134308 has been shown to be inactive or only weakly active in the displacement of the binding of radioligands to κ, μ, or δ opioid receptors (29). Analgesia has not been reported with other CCK receptor antagonists (16, 24, 25, 27, 28). It is possible that the antagonism by CCK of opioid analgesia operates to some extent tonically through the CCK-B receptor at the spinal level, and that blockade by PD134308 results in removal of this antagonism, leading to increased opioid peptide release.

We previously demonstrated that GAL given i.t. potentiated the effect of morphine on flexor reflex excitability and behavioral tests of nociception in a naloxone-reversible fashion (32). If the analgesic effect of PD134308 involves the endogenous opioid system one would predict that it should be potentiated by GAL as well. Indeed, the depressive effect of i.t. GAL, when combined with systemic PD134308, was reversed by naloxone and, in fact, was stronger than that after morphine at 1 mg/kg. Since we have previously shown that naloxone cannot reverse the reflex depression by a high dose of GAL (34), it appears as though the GAL receptor is independent of both the opioid and CCK-B receptors, but it can interact with them to cause a synergistic opioid-mediated analgesia. The synergistic interaction between GAL, PD134308, and morphine further strengthens this suggestion.

The site of the analgesic effect of PD134308 appears to be important mainly within the spinal cord, since we observed a similar effect in intact and, particularly, in spinalized rats. Furthermore, the interaction of PD134308 and morphine was also similar in intact and spinalized animals. Finally, the strongest argument for a direct spinal effect of systemic PD134308 is that it effectively antagonized the facilitatory effect of i.t. CCK and interacted with i.t. GAL to cause synergistic reflex depression. Previous experiments performed by us and others have documented a spinal site of interaction between CCK and opioids (15, 19, 20, 24). Moreover, extensive systems containing genuine CCK peptide (6–9) and CCK mRNA (35) are present in the rat dorsal horn, and there is also evidence for a descending CCK system to the spinal cord, originating in the mesencephalic central grey (36). However, CCK-B receptors also exist in many regions in the brain, including the periaqueductal grey (21, 22), known to be involved in opioid analgesia, and the interaction between CCK, its antagonists, and opioids has been reported after intracerebral or intracerebroventricular injection (15, 25). We can thus not rule out the possibility that the potentiation of morphine-induced analgesia on the hot plate test may be partially mediated by CCK-B receptors in the brain.

In conclusion, the present results demonstrate that a recently developed CCK-B antagonist in two experimental models can enhance the effect of morphine on analgesia and the nociceptive flexor reflex, especially when combined with the peptide GAL. Provided that similar mechanisms operate in the human spinal cord, our findings indicate new approaches to pain treatment based on combinations of drugs.

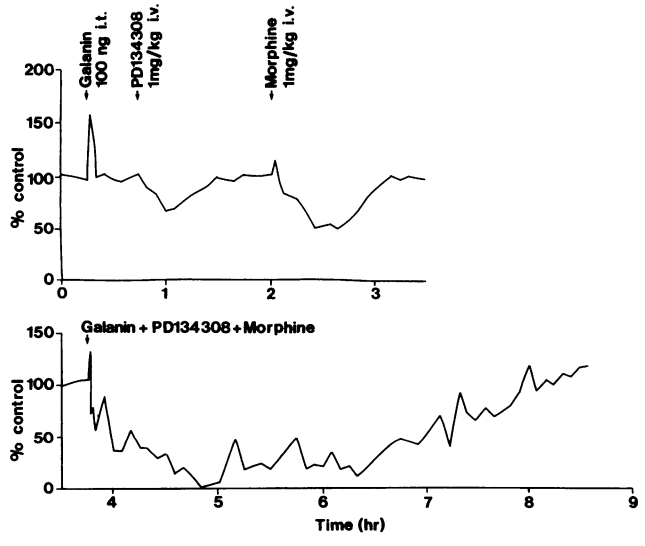

FIG. 7. Effect of GAL at 100 ng i.t., PD134308 at 1 mg/kg i.v., morphine at 1 mg/kg i.v. (*Upper*), and the combination of all three drugs (*Lower*; continuation of abscissa) on the flexor reflex excitability in a single experiment.

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