PHARMACOLOGICAL INTERACTION OF OPIATES WITH VARIOUS CLASSES OF CENTRALLY ACTING DOPAMINERGIC DRUGS

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SUMMARY

The comparative analgesic and sedative (narcosis potentiating) efficacy of mu and kappa opioids was studied as a function of time in rats and mice. The mu agonists, morphine and fentanyl, produced antinociceptive actions against both heat and chemical noxious agents, but the half-lives of their ED_{50}s were longer in the writhing than in the hot plate test. The kappa agonist drugs, bremazocine, ethylketocyclazocine and pentazocine, proved to be inactive against heat nociception, and produced a potent, long-lasting analgesia in the acetic acid writhing test, similar to mu agonists. The combination of two mu agonists resulted in a synergistic interaction and a remarkable prolongation of antinociceptive action. When the kappa-drug bremazocine was coadministered with morphine, there was a significant prolongation of the duration of analgesic action, without any influence on the potency. The interactions of mu and kappa opioids with agonists and antagonists at dopamine receptors were also studied in narcosis. The time course of the naloxone-morphine antagonism in analgesiometric assays revealed similarities, when apparent pA_2 values were estimated at the peak of agonist and antagonist activity, but it was different in the writhing test when the pA_2 was determined 84 minutes after morphine administration ($EDt_{½}$, half-life belonging to the ED_{50}) while naloxone was given at its peak effect.

KEY WORDS

opiate receptors, kappa opioid agonists, mu opioid agonists, neuroleptics, narcotics

INTRODUCTION

The opiate analgesics have been shown to have a variety of pharmacological effects, such as analgesia, sedation, euphoria, etc. In the mammalian central nervous system (CNS), multiple opioid receptors, namely mu, kappa, sigma and delta, have been implicated in the mediation of opioid antinociceptive and other activities /1-8/.

The analgesic effects of opiates have been explained by proposing that two separate receptors are responsible for mediating these effects: kappa opioid agonists have been observed to be potent analgesics, which fail to possess opioid-like side effects, such as dependence liability, respiratory depression and constipation /4,6,7/. The corresponding profile for agonism of mu receptors is characterized by analgesia, euphoria, constipation, tolerance and physical dependence capacity /1,5,6/.

The studies described in this report were carried out in an attempt to undertake a systemic evaluation of the comparative actions of mu and kappa opiates, mainly of their common activities, e.g., analgesia and sedation, and those of their possible therapeutic combinations, as a function of time. Although both mu and kappa opiate receptors mediate antinociception in animals, the interactions of analgesic drugs with these receptors may also be classified according to their antinociceptive activities against qualitatively and quantitatively different – heat or chemical – nociceptive stimuli. Drugs classified as mu agonists were equally effective against heat, pressure or chemical stimuli, while kappa agonists were inactive against heat nociception, and were effective against chemical and pressure induced pain /2,3/. Millan /9/ postulated that kappa opioid receptors can mediate antinociception against noxious thermal stimuli as well; however, in contrast to mu receptors, this antinociception against heat is intensity dependent, and kappa agonists are effective only for low intensity pain. Therefore, a different time course of pharmacological responses might be hypothesised, based not only on the heterogeneity of receptors, but also on differences between the nociceptive stimuli applied. Furthermore, a number of pharmacological similarities were found between neuroleptics and narcotics /10,11/: sedation (potentiation of narcosis), catalepsy, etc. Clay and Brougham /12/ reported competition for binding at selective brain sites between haloperidol and naloxone, suggesting that this neuroleptic binds to narcotic-specific receptors. Although sigma sites were initially defined as "sigma-opioid" receptors /1/, the failure of opioid antagonists, such as naloxone, to antagonize sigma

pharmacological effects indicates that they are not classical opioid receptors /13/. Drugs having affinity for the sigma-binding sites (e.g., cyclazocine, pentazocine, phencyclidine) have psychotomimetic effects, while certain non-opioid agents have high affinity for the sigma binding site, producing anti-psychotic activity. The "haloperidol sensitive" sigma sites may mediate the psychotomimetic effects of certain opiates, and, possibly, the antipsychotic activity of neuroleptic drugs. Thus, haloperidol, or several dopaminergic drugs (such as phenylpiperidines, butyrophenones and phenotiazines) exhibit affinity for sigma binding sites, which might suggest a functional link between dopaminergic receptors and sigma receptor binding sites /14/. In addition, sigma opioid compounds (SKF-10,047; cyclazocine) have been observed to influence dopamine metabolism and neurotransmission /15/.

Yonehara and Clouet /16/ distinguished the effects of prototype receptor agonists on dopamine release: morphine and delta ligands were more effective than kappa agonists in increasing the turnover of striatal dopamine. Barasi *et al.* /17/ observed dopamine receptor mediated nociception in normal and haloperidol treated rats.

Another purpose of our investigation was to analyse the nature of the central depression, elicited by various types of opioids and certain neuroleptics.

MATERIALS AND ANIMALS

Materials

We are most grateful to the following for gifts of drugs: morphine hydrochloride, pentazocine, apomorphine hydrochloride (Alkaloida, Hungary); bremazocine (Sandoz); ethylketocyclazocine, EKC (Sterling-Winthrop Res. Int.). The other drugs used were purchased: bromocriptine (Sandoz); fentanyl, haloperidol, reserpine (Richter-Gedeon, Hungary); naloxone (Du Pont); chlorpromazine (Egis, Hungary); amphetamine (Chinoin, Hungary); inactin: ethyl-buthyl-thiobarbiturate-Na (Byk.Gulden, Hamburg). The drugs were administered subcutaneously (s.c.) or intravenously (i.v.) to rats and mice in a volume of 5 ml/kg and 10 ml/kg body weight, respectively. Intracerebroventricular (i.c.v.) injections were administered to rats, in a volume of 10 μl/animal.

Animals

Groups of five male and female Sprague-Dawley rats weighing 120-150 g and groups of ten mice of both sexes weighing 20-25 g

were used. The animals were kept in the departmental animal house for at least one day prior to the experiment. They were allowed free access to standard laboratory diet and tap water, and were kept in artificial lighting for 12 h each day. The room temperature was 22 ± 2°C.

METHODS

Analgesiometric assays

Hot plate test (rat)

The original method of Woolf and McDonald was used /18/. Hot plate latencies were determined by placing each rat on a hot plate kept at 55±1°C and observing the occurrence of nociceptive responses: licking of the paw or jumping. Each animal served as its own control. The arbitrary cut-off time was 2.5 times the control reaction time and was taken as 100%.

Tail flick test (rat)

The original method of D'Amour and Smith /19/ was used to determine analgesia in the rat by measuring the time required to respond to a painful radiating heat stimulus. An initial reaction time was first established (control) and the time to withdrawal of the tail (using an arbitrary cut-off time twice the control reaction time) was determined and compared to the postdrug reaction time and expressed as a percentage.

Acetic acid writhing test (mouse)

The writhing assay was used in mice according to the method of Hendershot and Forsaith /19/. Mice were injected intraperitoneally with 0.2 ml of a 0.6% acetic acid solution to produce the writhing reaction. The number of writhes per animal was counted during a five min period. The analgesic activity was expressed as the percentage inhibition of the average number of writhes in control animals on the same day.

Narcosis potentiation (rat)

This effect was determined by measuring the sleeping times in a group of 10 male rats. Inactin was injected at 35 mg/kg into the tail

vein. The times at which animals lost and regained their righting reflex were recorded.

Determination of the *in vivo* equivalent of pA_2: apparent pA_2

The pA_2 of a competitive antagonist was defined by Schild /21/ as the negative logarithm of the molar concentration of an antagonist, which reduces the effect of a dose of agonist to that of half dose. This concept has been used to characterize analgesic receptors *in vivo* /22-25/. According to Schild's concept, similar pA_2 values could be expected in various assays when using a single agonist/antagonist combination, if the drugs interact with a similar type of receptor, since pA_2 values reflect the affinity constant of the antagonist to the receptor. In our experiments the *in vivo* equivalent of pA_2 was determined for morphine-naloxone in various analgesiometric assays: hot plate, tail flick (in the rat), writhing test (in the mouse) and in narcosis potentiation. The dose-response curves were determined for morphine alone and in combination with three different doses of naloxone, either at the time of the peak effect of both drugs, or during the offset of the analgesic effect of morphine. For each shift in the dose-response curve, the ratio of the ED_{50} of the morphine in the presence of naloxone to the ED_{50} of the drug alone (dose ratio, DR) was calculated:

$$DR = \frac{ED_{50} \text{ morphine + naloxone}}{ED_{50} \text{ morphine alone}}$$

-Log doses of naloxone in moles/kg were plotted against log(DR-1), and fitted to a straight line. The intercept on the abscissa gives the apparent pA_2 value.

RESULTS AND DISCUSSION

The analgesic actions of different opiate receptor agonists as a function of time

In the course of our study we accepted a current concept in pharmacology, namely, that a direct and reversible pharmacological effect is associated with a particular drug concentration at the site of action /26/; thus we considered the time-response curve as an approximate expression of drug concentration.

A comparison of the median antinociceptive activities of mu and kappa opioids

The analgesic activities (ED_{50}) of opiate agonists were determined by different methods and in different species, applying heat (hot plate, tail flick, rat) and chemical irritation (acetic acid writhing test, mouse) as nociceptive stimuli, since it was observed that mu and kappa opiates were differentially sensitive /2,3,9/.

All agents tested produced dose-dependent antinociception in the writhing test, while only mu agonists were active both in the hot plate and tail flick, or acetic acid writhing tests (Table 1). Benzomorphans (bremazocine, ethylketocyclazocine, pentazocine) showed dose-response correlations in the hot plate test but, in contrast to the effects of morphine, the effective doses of these drugs also produced marked motor impairment, making animals unable to respond to the painful stimulus. The activity of bremazocine, a kappa agonist drug, proved to be thirty-fold higher than that of morphine, and five-fold that of ethylketocyclazocine, and it was as potent as fentanyl, a selective mu agonist, in the mouse writhing test.

A comparison of the half-lives of the analgesic ED_{50} ($EDt_{½}$) of mu and kappa agonist opioids

The time course of the antinociceptive effects of mu agonists against heat nociceptive stimulus (hot plate test) – For the selected opiate analgesics, the correlations between time and effect were demonstrated and compared as $t_{½}$ of the ED_{50} values ($EDt_{½}$) of the particular drug (Figures 1-5). As apparent from Figures 1-3, the time course of morphine declined more slowly when its effect was measured in the mouse writhing test (Fig. 3), than in the rat hot plate test (Fig. 1 for s.c., Fig. 2 for intracerebroventricular administration) when the $EDt_{½}$ values were compared: 84 min, 47 min and 35 min, respectively. Similar observations were made with another mu agonist, fentanyl, using the same assays: half-lives measured for fentanyl were 52 min (writhing test) and 36 min (hot plate test) respectively (Figs. 4, 5).

The time course of the antinociceptive effects of kappa agonists against chemical (acetic acid) nociceptive stimuli (writhing test) – Since the kappa agonist analgesics, such as ethylketocyclazocine (EKC), bremazocine and pentazocine, were found to be essentially inactive against heat nociception, although they showed definite dose-response correlations in the writhing test, it was possible to

TABLE 1

The antinociceptive activities of some mu and kappa opiate agonists

	ED_{50} (mg/kg, s.c., 95% confidence limit)					
	hot plate	N	tail flick	N	writhing test	N
mu agonists						
morphine	3.6 (2.57-5.04)	30	1.8 (1.0-3.1)	20	0.69 (0.53-0.89)	20
fentanyl	0.048 (0.032-0.072)	15	0.024 (0.021-0.028)	20	0.036 (0.025-0.050)	10
kappa agonists						
bremazocine	–		–		0.024 (0.012-0.043)	10
ethylketocyclazocine	–		–		0.12 (0.075-0.19)	10
pentazocine	–		–		8.1 (5.4-12.3)	10

– inactive

N number of animals at each dose level.

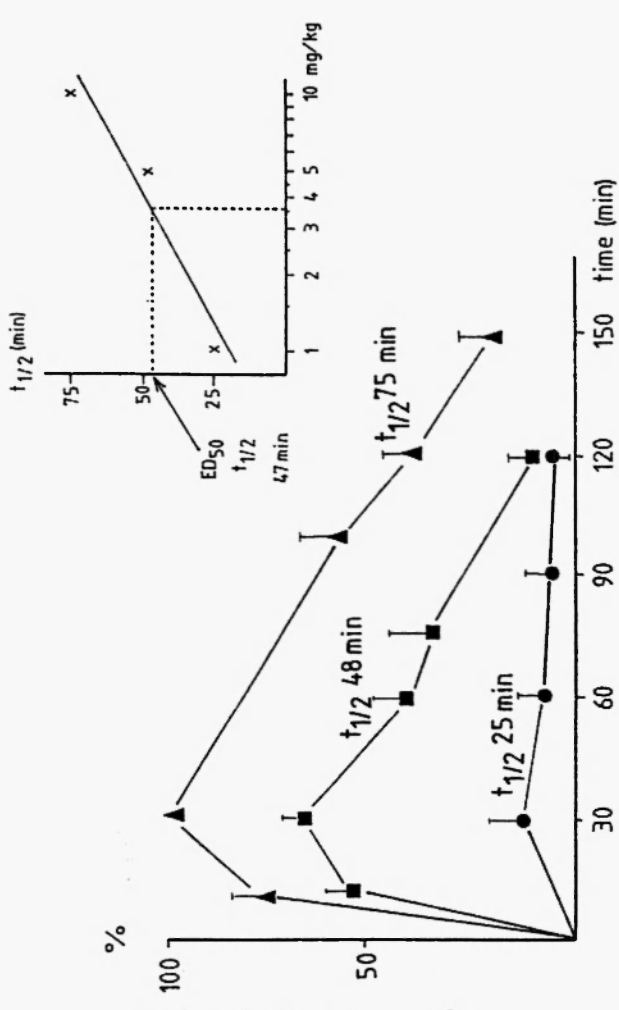

Fig. 1: Time course of the analgesic effect of morphine, administered subcutaneously (s.c.) to the rat, in the hot plate test. The doses were: 1 mg/kg (●), 5 mg/kg (■) and 10 mg/kg (▲). Mean ± SEM depicted, n=10 (15) per point. In the top right panel the solid line represents the curve constructed by plotting $t_{1/2}$ values against the increasing doses of morphine ($t_{1/2}$ is considered as the time elapsed between the peak activity and its decrease to the half, expressed in min). $t_{1/2}$ belonging to the ED_{50} is graphically evaluated

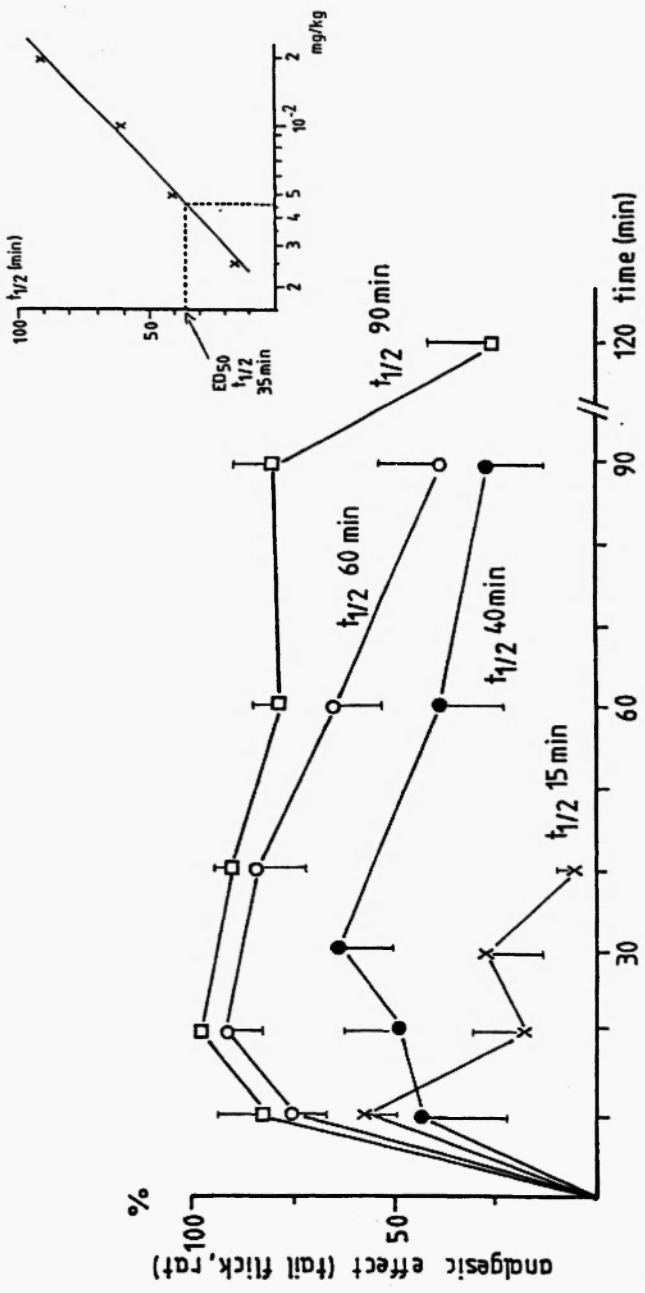

Fig. 2: Time course of the analgesic effect of morphine, administered intracerebroventricularly to the rat, in the tail flick test. The doses were: 2.5 µg/rat (X), 5.0 µg/rat (●), 10 µg/rat (○), 20 µg/rat (□). For calculation of $t_{½}$ see legend of Fig. 1.

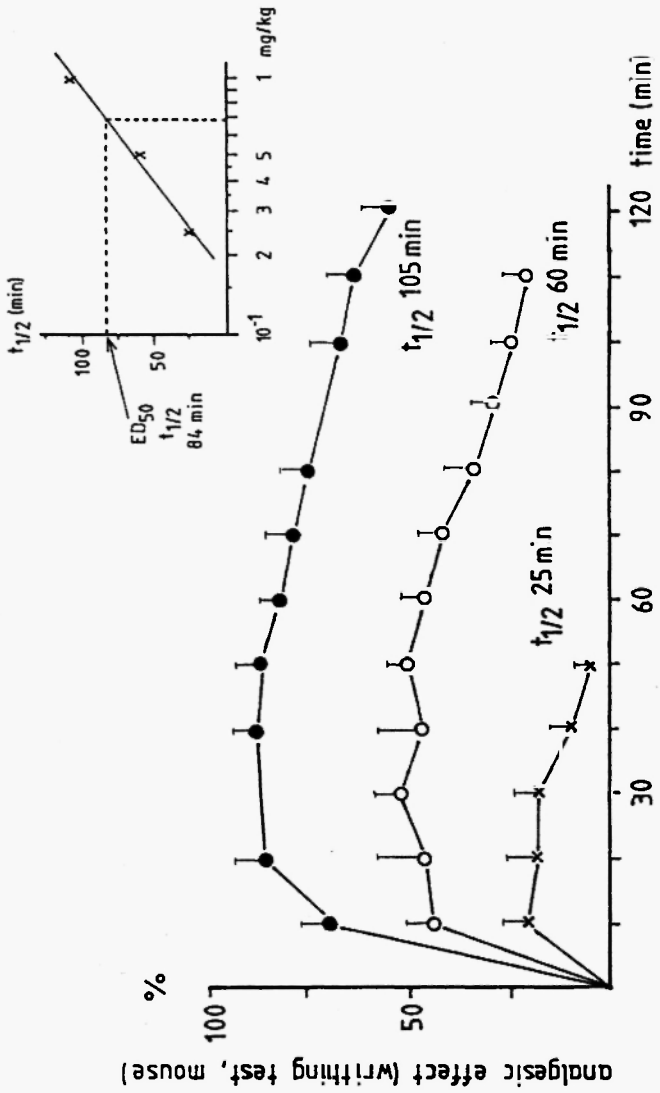

Fig. 3: Time course of the analgesic effect of morphine, administered s.c. to mice, in the writhing test. The doses were: 0.25 mg/kg (X), 0.5 mg/kg (○), 1.0 mg/kg (●). For calculation of $t_{1/2}$ see legend of Fig. 1.

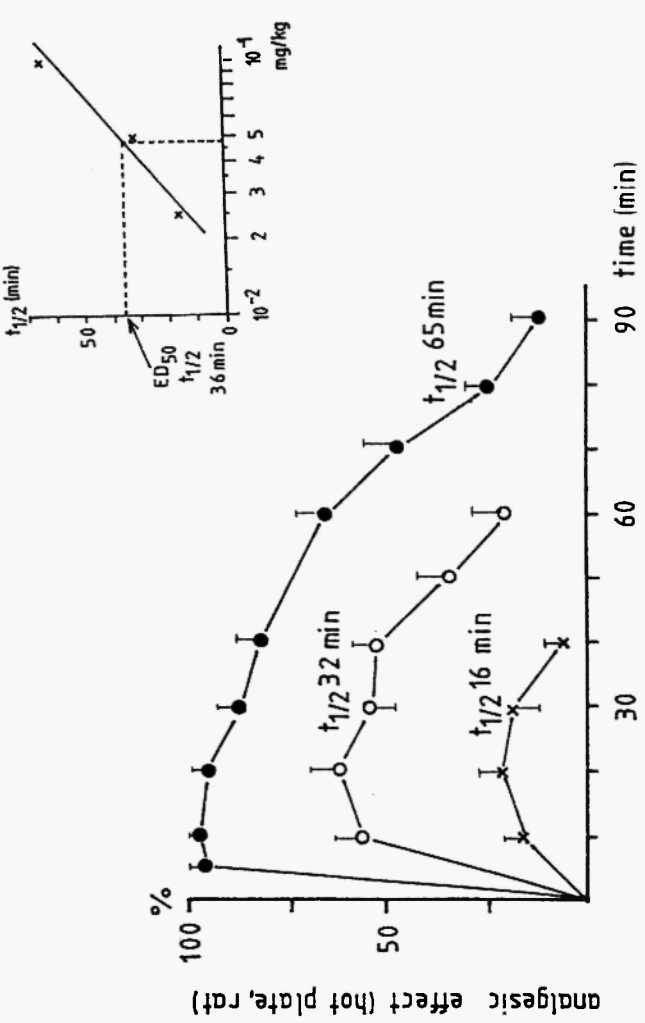

Fig. 4: Time course of the analgesic effect of fentanyl, administered s.c. to the rat, in the hot plate test. The doses were: 0.025 mg/kg (X), 0.050 mg/kg (○), 0.100 mg/kg (●). For calculation of $t_{1/2}$ see legend of Fig. 1.

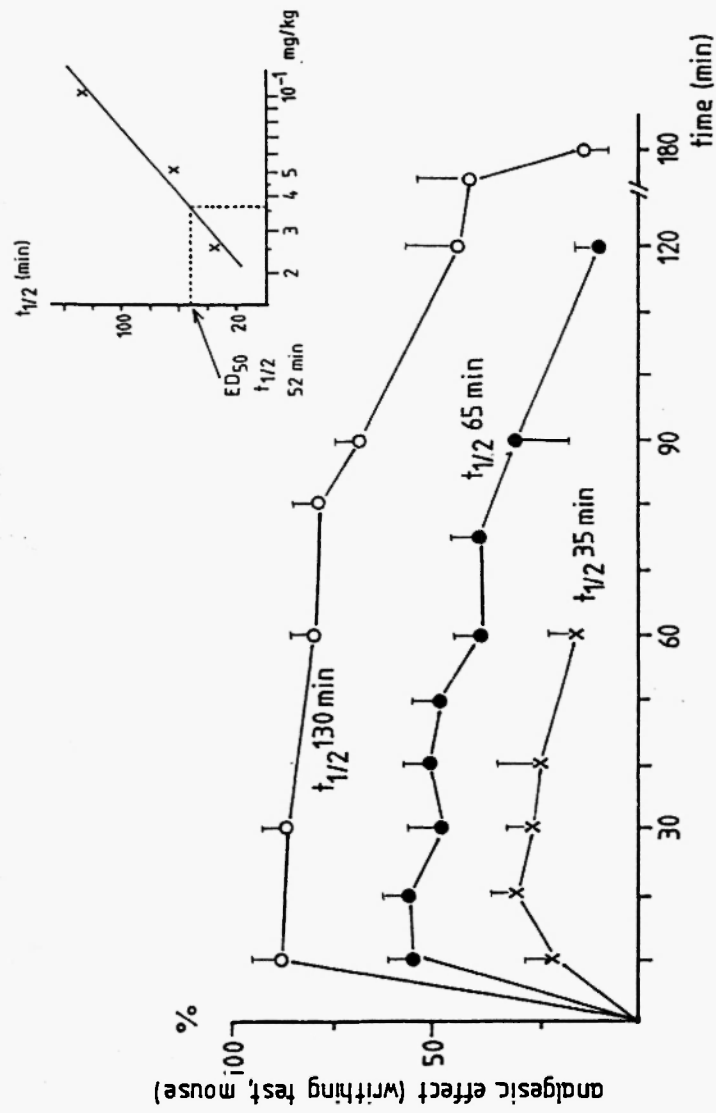

Fig. 5: Time course of the analgesic action of fentanyl, administered s.c. to mice, in the writhing test. The doses were: 0.025 mg/kg (X), 0.050 mg/kg (●), 0.100 mg/kg (○). For calculation of $t_{½}$ see legend of Fig. 1.

compare their analgesic activity as a function of time in only the latter assay (Table 2).

The kappa agonists produced time-response curves and $ED t_{½}$ values similar to those produced by mu agonists (Figs. 3, 5) in the mouse writhing test.

TABLE 2

The time course of the analgesic effect of kappa agonists measured in the mouse writhing test

compound	dose mg/kg s.c.	time after drug administration (min) 10	20	30	60	90	120	ED_{50} $t_{½}$ (mm)
		(% analgesia)						
penta-	5.0	20	18	22	20	13	10	
zocine	10.0	40	48	54	49	38	22	
	15.0	48	62	76	70	68	55	58
EKC	0.050	38	45	40	39	32	18	
	0.100	49	60	58	48	41	29	
	0.250	67	80	78	79	68	40	79
brema-	0.05	66	-	56	51	25	-	
zocine	0.10	88	-	85	75	51	2	
	0.25	100	-	100	88	75	23	64

The number of animals at each dose level was 10.
- not tested

The time course of the antinociceptive effects of the combinations of mu and kappa agonists measured in rats and mice, in the hot plate and writhing tests, respectively – Table 3 demonstrates the time course ($t_{½}$) of the antinociceptive actions of various combinations of mu and kappa agonists in the hot plate (rats) and writhing (mice) tests. In the upper part of the Table, the effects of drugs are presented alone, either in low ineffective (e.g., morphine, 0.25 mg/kg, in the writhing test, or 1.0 mg/kg in the hot plate test; fentanyl, 0.025 mg/kg in both tests, etc.) or submaximal doses (e.g., morphine, 10 mg/kg in the hot plate test, or bremazocine, 0.1 mg/kg in the writhing test), according to their participation in the combinations (lower part). The data for the combinations of drugs belonging either to identical (mu + mu) or different (mu + kappa) classes of opiates are shown. The coadministration of subactive doses of two mu agonists (morphine + fentanyl) resulted in a synergistic interaction, which seemed to surpass the theoretical additive interaction in both analgesic tests; in addition, the duration

of their antinociceptive actions was also remarkably prolonged. However, when subactive doses of either two kappa agonists, or a kappa agonist + a mu agonist were combined, no significant changes in analgesic action could be observed in the hot plate test, while an additive interaction was observed in the writhing test. Interestingly, when a submaximal dose of morphine (10 mg/kg) was combined with an inactive dose (0.1 mg/kg) of bremazocine, although the latter drug did not influence the potency of the analgesic action of morphine, the prolongation of the duration of its action was seen, even in the hot plate test, in which the kappa drug alone failed to produce an analgesic effect in the rat.

TABLE 3

Changes in potency and duration of the analgesic action produced by combinations of differently classified opioids

Drug	dose mg/kg s.c.	analgesia (%) hot plate (rat)	analgesia (%) writhing (mouse)	$t_{1/2}$ (min) hot plate (rat)	$t_{1/2}$ (min) writhing (mouse)
Alone:					
morphine	0.25	-	12	-	-
morphine	1.0	12	-	-	-
morphine	10.0	98	-	75	-
fentanyl	0.025	18	27	16	15
pentazocine	2.5	12	22	25	-
bremazocine	0.01	5	24	-	-
bremazocine	0.1	22	88	-	75
In combination:					
morphine + fentanyl	0.25+ 0.025	-	65	-	70
morphine + fentanyl	1.0+ 0.025	85	-	130	-
bremazocine + pentazocine	0.101+ 2.5	-	51	-	35
bremazocine + pentazocine	0.1+ 2.5	38	-	-	-
bremazocine + morphine	0.01+ 0.25	-	48	-	-
bremazocine + morphine	0.1+ 1.0	28	-	25	-
morphine + bremazocine	10.0+ 0.1	88	-	130	-

The number of animals for each dose level was five (hot plate) or ten (writhing test).
- not tested

It is noteworthy that the duration of the antinociceptive activity produced by a combination of morphine with fentanyl or bremazocine, was significantly longer than that caused by the highest single dose of morphine (see Fig. 1).

Interactions of opiates and dopaminergic drugs in barbiturate induced narcosis

All the agents tested, dopamine agonists (apomorphine, bromocriptine) and dopamine antagonist neuroleptic agents (chlorpromazine, haloperidol, reserpine), produced dose-dependent prolongation of barbiturate-induced sleeping, similar to mu and kappa agonist opiates (morphine, fentanyl and EKC; bremazocine and pentazocine) (Fig. 6). The dose-response curves were determined at the time of peak effect.

Reserpine

The narcosis potentiating action of 2.5 mg/kg reserpine as a function of time is shown in Fig. 7. The effect of 2.5 mg/kg reserpine was gradually increased from ½-4 hours of pretreatment, while the effect returned to the control level after 16-24 hours preadministration. In addition, the time-response curve of the narcosis potentiating effect of the submaximal dose (2.5 mg/kg) of morphine is shown in Fig. 8. Morphine reached its peak effect when it was given 30 minutes prior to the base-barbiturate while there was a gradual decline in its activity ½, 1, 2, 4 or 24 hours after its administration.

However, when morphine was coadministered with reserpine, their actions were found to be additive between 2 and 4 hours of reserpine pretreatment, but it resulted in complete inhibition of morphine action when reserpine was administered 24 hours prior to morphine. These observations differ significantly from that for analgesic action: pretreatment with reserpine at various intervals failed to influence the analgesic action of both mu and kappa agonists, while the narcosis potentiating effects of the latter were also prevented by 24 hours pretreatment with reserpine (Fig. 9). Reserpine depletes stores of catecholamines and 5-HT in many organs, including brain, within an hour after its administration, and the depletion is maximal by 24 hours /27/. These effects could explain the characteristic sedation and state of indifference produced by reserpine. It is, therefore, clear that reserpine interferes with the intracellular storage of endogenous transmitters and modulators. One might speculate that such changes at 24 hours

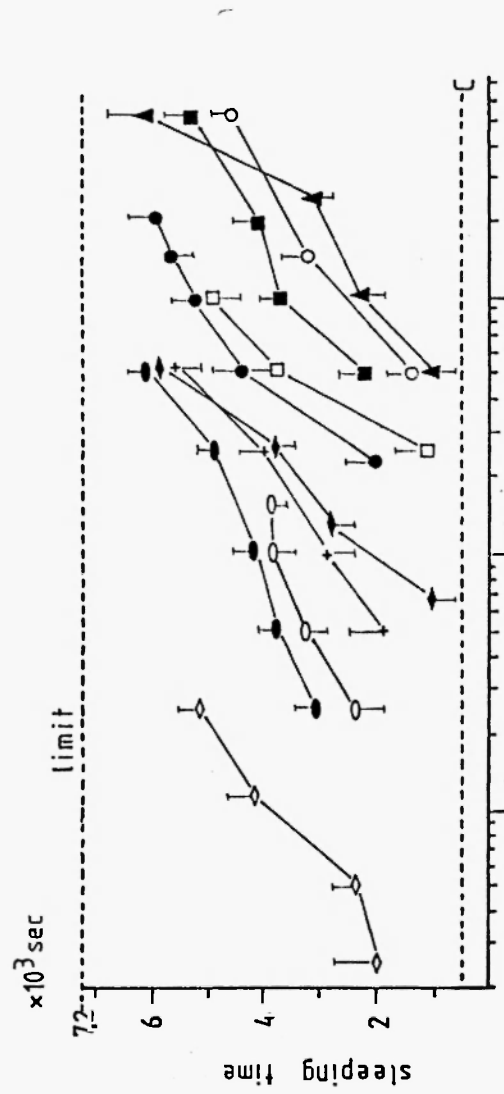

Fig. 6: Influence of various centrally acting agents, administered so to the rat, upon sleeping time induced by inactin (ethyl-butyl-thiobarbiturate). Sleeping time is expressed in sec (ordinate) and limited to two hours, as demonstrated by the upper dotted line, plotted against dose (abscissa). The average of the control (C) narcosis time is demonstrated by the lower dotted line. Fentanyl (◇), bemizocine (●), ethylketocyclazocine (⊂), chlorpromazine (+), haloperidol (◆), reserpine (●), apomorphine (□), pentazocine (■), morphine (▲), bromocriptine (○).

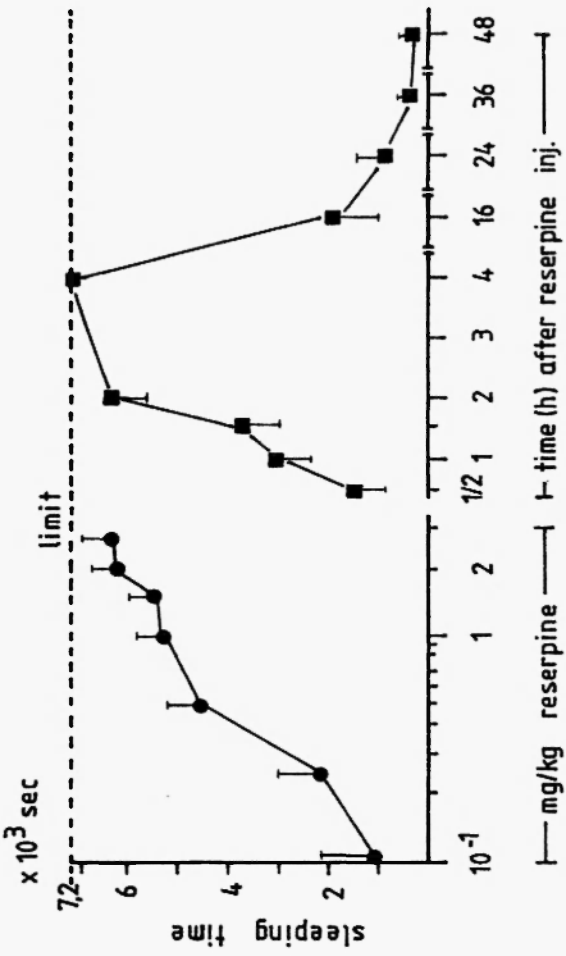

Fig. 7: The narcosis potentiating effect of reserpine, administered s.c. to the rat, as a function of time. Dose response curve of reserpine (●) taken at 2 hours after administration. Time-response curve of 2.5 mg/kg reserpine (■). Note the peak effect observed between 2-4 hours pretreatment.

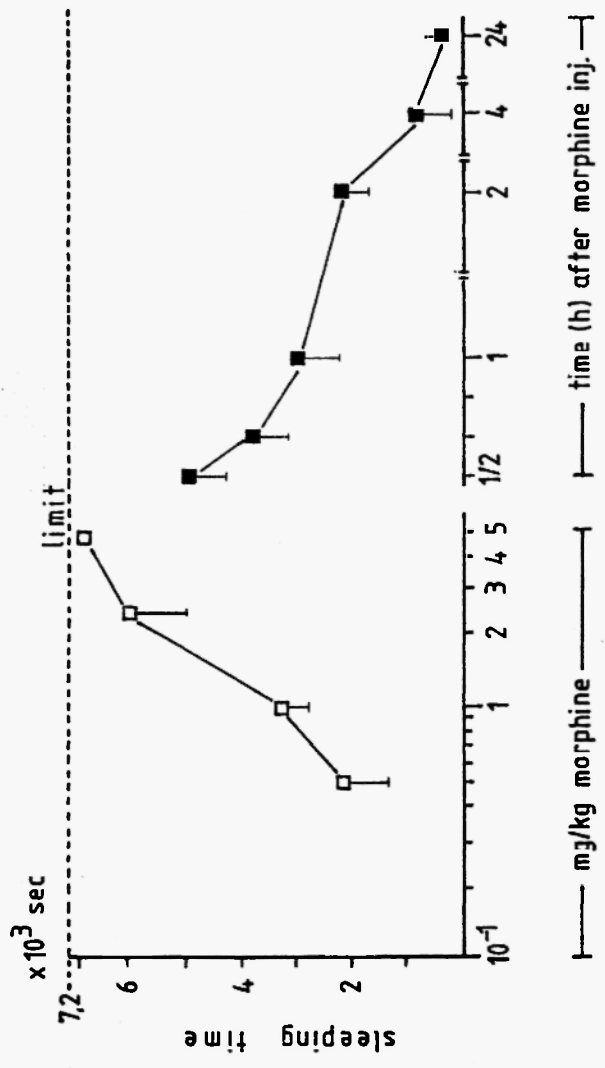

Fig. 8: The narcosis potentiating effect of morphine, administered s.c. to the rat, as a function of time. Dose response curve of morphine (□). Time-response curve of 2.5 mg/kg morphine (■).

Fig. 9: The influence of various intervals of reserpine pretreatment (2, 4 and 24 hours) on the narcosis potentiating effects (■) of mu (morphine) and kappa (tiflamazocine) opioid agonists, and upon morphine analgesia (hot plate, □). C-1 = control narcosis time; C-2 = 5 mg/kg morphine s.c., hot plate A = 2.5 mg/kg reserpine; B = 2.5 mg/kg reserpine + 2.5 mg/kg morphine; C = 2.5 mg/kg reserpine + 0.5 mg/kg bremazocine; D = 2.5 mg/kg reserpine + 5 mg/kg morphine; hot plate
Each column corresponds to the mean sleeping time or percent analgesia measured in the hot plate test. Vertical bars indicate standard error of the mean. Note the ineffectiveness of reserpine to influence the analgesic effect of morphine, by 24 hours pretreatment, when the inhibition of narcosis potentiating effects of either morphine or bremazocine was complete.

might have resulted in endogenous opioid peptide depletion as well, explaining the inhibitory action of reserpine against morphine narcosis potentiating effect, since there are cells in the CNS which are reported to contain either enkephalins or 5-HT /28/. The lack of inhibition of morphine analgesia by reserpine can be explained by the fact that different receptors might be involved in the sedative and analgesic actions of opiates; the former seems to be mediated better with kappa or delta than with mu opioid receptors. In addition, the neural systems that mediate opioid sedation appear to be distinct from those involved in the classical, mu-type opiate analgesia. The depletory effect of reserpine on opioid containing neurons requires further research.

Dopamine agonists (apomorphine, bromocriptine, amphetamine) and antagonists (haloperidol, chlorpromazine)

Similar results were obtained by reserpine pretreatment when barbiturate narcosis was prolonged by either the dopamine agonists, apomorphine and bromocriptine, or the dopamine antagonists, chlorpromazine and haloperidol (Fig. 10). In contrast to the potentiating effect of 2 or 4 hours pretreatment by reserpine, a complete abolition of sleeping was observed by 24 hours pretreatment. Amphetamine, the dopaminomimetic drug, did not alter the sleeping time significantly, although the control narcosis time was slightly decreased. However, amphetamine was found to shorten significantly the duration of narcosis, potentiated either by dopamine agonists or antagonists, or by mu or kappa opiate agonists (Figs. 9, 10). Since barbiturates – like opiates or neuroleptics – are CNS depressants, and amphetamines are central stimulants, they generally produce opposite effects. Schechter /29/ observed an antagonism between amphetamine and pentobarbitone in rats trained to discriminate between the subjective behavioural effects of amphetamine and pentobarbitone, and suggested a common mechanism or site of action. Our earlier observation, that naloxone shortened the narcosis time evoked by pentobarbitone, and diminished the toxicity of the latter, also seems to support this hypothesis /30/. As previously reported, the narcosis lengthening effects of either opiates or chlorpromazine and apomorphine were abolished by naloxone, whereas the latter drug failed to shorten significantly this action of haloperidol or bromocriptine /31/.

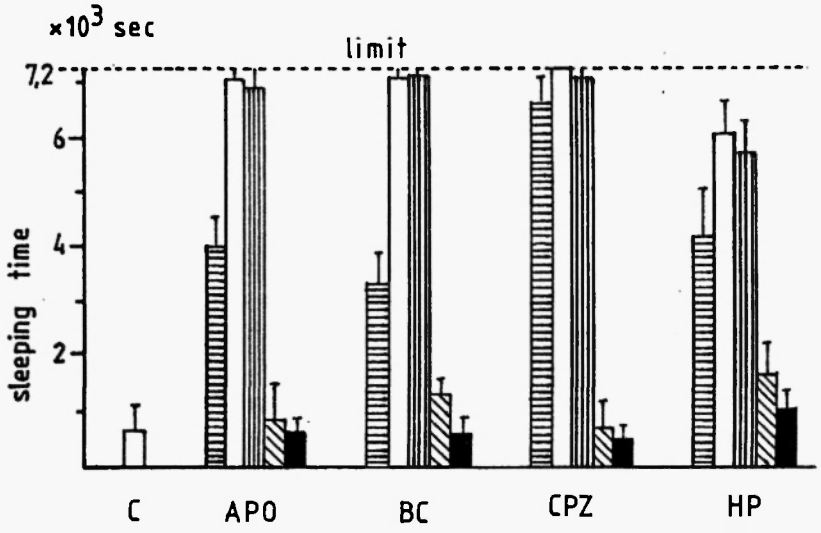

Fig. 10: The influence of various intervals of reserpine pretreatment (2, 4 and 24 hours) and coadministration with amphetamine upon the narcosis potentiating effects of apomorphine, 1.0 mg/kg (APO); bromocriptine, 1.5 mg/kg (BC); chlorpromazine, 0.5 mg/kg (CPZ); haloperidol, 0.5 mg/kg (HP). C=control narcosis time, saline pretreatment (▤); reserpine pretreatment, 2 hours (□); 4 hours (▥); 24 hours (▨). Coadministration with amphetamine, 2.5 mg/kg (■). Note the identical inhibitory actions of amphetamine and 24 hours reserpine pretreatment upon sleeping time prolonged by pharmacologically divergent groups of drugs.

Antagonism of analgesic and sedative (narcosis potentiating) actions (pA_2: time course and pA_2)

The determination of *in vivo* pA_2 ("apparent pA_2") was used to classify receptors involved in various analgesiometric assays and in narcosis.

Several authors /22-25/ have applied this method to experiments conducted on intact animals, using administered doses for agonists and antagonists on the assumption that, in such situations, the concentrations at the receptor sites are proportional to the administered doses. Because the concentrations are time-dependent functions, measurements were made at the instant of the peak effect, a time at which the drug would most likely be in

equilibrium with the receptor. Since it is unlikely that the agonist and the antagonist would reach their peaks at the same time interval after their administration, the schedule of dosing must be such that both drugs are at their maximum tissue concentrations at the time of measurement. It is only at this time that the proportionality can hold. We have applied this time-dependent method to the analysis of data utilizing morphine and naloxone with the hot plate, tail flick writhing test, and narcosis (Fig. 11). In our experiments the agonist morphine reached its maximal effect at 30 min after its administration, while the antagonist, naloxone, at 15 min after its administration. The dosage schedule was as follows: morphine was administered at t=0; naloxone at t=15 min;

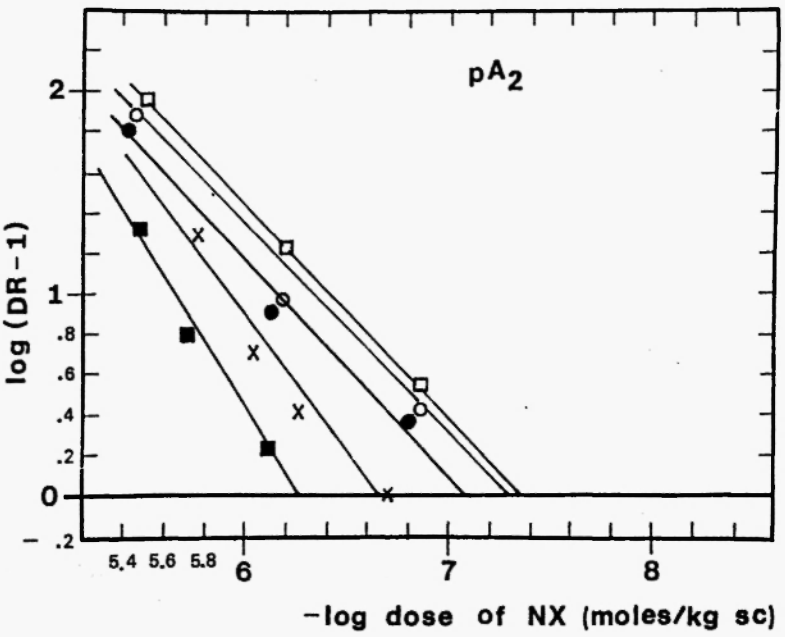

Fig. 11: Estimation of the apparent pA_2 for naloxone-morphine, using tail flick, pA_2=7.38 (□), hot plate, pA_2=7.35 (○) and writhing assays, pA_2=7.1 (●), narcosis-potentiation, pA_2=6.7 (X) at peak effects of drugs. Estimation at $EDt_{\frac{1}{2}}$ of morphine ($EDt_{\frac{1}{2}}$: half life of the analgesic effect of morphine belonging to the ED_{50} value) in the writhing test: 84 min, pA_2=6.35 (■). Log (DR-1) is plotted against -log dose of naloxone (NX) moles/kg, s.c. For details see Methods.

measurement was made at t=30 min. The importance of making measurements at the time of peak effect has been stressed by Takemori *et al.* /25/. The effect diminishes after a given time, presumably because the agonist concentration has decreased. Furthermore, the magnitude of the shift and, hence, the dose ratios (see Methods) also decrease with time, since concentration of antagonist would also decrease. It was therefore of interest to estimate pA_2 after the peak effect of the agonist, namely at $EDt_{½}$ of morphine: 84 min (see Fig. 3) in the writhing test. According to this protocol, the antagonist (naloxone) was given at its peak time (15 min).

In spite of the different potency of morphine in the analgesiometric assays (see Table 1), the apparent pA_2 values for morphine - naloxone were very similar: 7.38 and 7.35 when they were estimated against noxious heat (hot plate and tail flick test, in the rat) while receptor affinity seemed to decrease in the mouse, against noxious chemical stimuli (writhing test): $pA_2 = 7.1$, taking measurements at estimated peak effects. These data are in good agreement with those determined by others /22-24/. A higher amount of naloxone was required to antagonize the narcosis potentiating effect of morphine: $pA_2 = 6.7$, when measuring at its peak effect. When the pA_2 value was estimated at 84 min after morphine administration, the lowest value was obtained: 6.35 (Fig. 11). It is possible that when morphine starts to dissociate from its receptor in the presence of naloxone, which displaces it very actively, at the latter's peak effect, morphine might begin to combine with another receptor subtype, which might be less sensitive to naloxone antagonism.

These results suggest that the affinity of the antagonist to its receptor was very similar when heat was the noxious agent, in the rat, supporting the observations made by several authors /2-4,9/, when measurements were made at the time of peak effect, and this value seems to be close to that determined in the chemical irritation test in the mouse, suggesting an interaction with another receptor subtype, possibly a kappa receptor.

As regards the barbiturate induced narcosis, it might be mediated by a different type of receptor, which would explain the lower pA_2 value (6.7). Nevertheless, when the potentiation of barbiturate-induced sleeping is investigated, the role of a number of factors other than the opioid receptors should also be taken into account: depression of CNS, hypothermia, inhibition of drug-metabolizing enzymes, redistribution, an increase of sensitivity of CNS to barbiturates, and so on. Most centrally active drugs, such as chlorpromazine, diazepam, alprenolol, fenfluramine, etc., prolong

the barbiturate-induced sleeping time by acting on the CNS and/or on the liver metabolism of the hypnotic /32,33/.

CONCLUDING REMARKS

1. It is advisable to take into account the kinetics of heterogeneous receptors, and the factors of nociceptive stimuli of various intensity, when the antinociceptive activity of opiates is evaluated.
2. When depression of the CNS is induced by different agents, it is important to consider the influence of different simultaneously activated receptors, e.g., opioid, dopamine, sympathetic, etc.
3. When the antagonism of a particular receptor action is examined, it is highly dependent on the time elapsed between the coupling and the dissociation from the binding site since, during dissociation from the receptor, the sensitivity and affinity might change to a particular receptor, or the selectivity might increase to another class of receptor.

ACKNOWLEDGEMENTS

Mrs. I. Wachtl, Miss K. Parina and Mr. S. Peter are thanked for excellent technical assistance.

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