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Analgesic effect of tramadol in the rat

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ABSTRACT The analgesic effect of tramadol was studied in the rat using tail flick and hot plate tests following intrathecal and subcutaneous administrations. Tramadol had only a short-lasting analgesic effect (20 min) on intrathecal administration which may be due to rapid removal from the subarachnoid space. Its analgesic effects were antagonized by pretreatment with naloxone. It seems that the opiate system may involve in the analgesic effect of tramadol, while the noradrenergic, serotonergic and cholinergic systems may play a modulating role.

KEY WORDS tramadol; opium; subcutaneous injections; spinal injections; analgesia; pain.

Tramadol is a centrally acting analgesic with weak opioid like properties. It has a negligible depressant effect on the respiratory system and causes no physical dependence[4,5]. The analgesic activity is less powerful than morphine and its allied drugs. Although its analgesic effects are antagonized by pretreatment with naloxone, it appears that its affinity to opiate receptors is low in comparison to morphine (personal communication Grunenthal). Recently, tramadol has been introduced into anaesthetic practice[11,12] with reasonably good analgesic effect. Numerous studies have provided evidence that the analgesic effect of opiates is mediated by the noradrenergic[7], serotonergic[9,10] and cholinergic systems[1,3,6].

The present investigation was designed to test whether tramadol had similar effects on these systems. The analgesic effect of tramadol was studied in the rat using tail flick and hot plate tests following intrathecal and subcutaneous administration.

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MATERIALS AND METHODS

Experiments were performed in Wistar rats (219 ± SD 15 g) of either sex. For intrathecal administration of the drugs the subarachnoid space in the rats was catheterized, as described in detail elsewhere[2]. First the rats were anaesthetized with halothane (1-2%) and N_2O in O_2 (2:1). Then a thin polyethylene catheter (o. d. 0.61 mm) was introduced into the subarachnoid space via the cisterna magna. It was advanced caudally for 8 cm so that the tip lay in the lumbar region. It was then filled with sterile saline solution and sealed. The rats were housed individually with free access to water and food. They were allowed to recover (5-7 d) and only those showing no neurological abnormality were used in subsequent experiments.

Nociceptive tests To evaluate the analgesic effect of the drug, the latencies of the rats reactions was measured using hot plate and tail flick tests. These measurements were determined both before and after drug or, in the case of the controls after the saline pretreatment.

1 Tail flick test: The tail flick response was thermally evoked by placing caudal part of the tail over a slit through which radiant heat from a halogen infrared reflector lamp (Osram, The Netherlands) was focused. The intensity of the heat was adjusted so that the rats flicked the tail within 3-5 s. The cut-off time was set at 30 s in order to prevent tissue damage.

2 Hot plate test: A fibreglass cylinder (15 cm diameter, 30 cm high) with a metal base was used for the hot plate test. The cylinder was placed in a hot bath and the

temperature of the metal surface was maintained at 55℃. After the tail flick test the rats was placed in the cylinder. The response latency was taken as the time between placing the rats on the metal surface and the licking of the hind paw, or jumping. In the absence of a response the cut-off time was set at 60 s.

Drug adminstration All drugs were injected intrathecally in volumes of 5-10 μl and this was followed by 10 μl of saline solution to flush the catheter. For systemic application, the drug or in the case of the controls sc of saline solution 1 ml/kg were applied. The tail flick and hot plate latencies were determined 10, 20, 40 and 60 min after the administration of the drug or saline. All drug solutions were freshly prepared in physiological saline solution.

Drug used Tramadol[(*d*,*l*)1-methoxyphenyl)-2-(dimethylamino-methyl)-cyclohexan-1-ol] (Grunenthal, F R Germany); ketanserin tartrate (Janssen Pharmaceutica, Belgium); naloxone (Du Pont, The Netherlands); physostigmine salicylate (Pharmacy, Erasmus University, The Netherlands); phentolamine methane sulphate(Ciba-Geigy, The Netherlands); methysergide hydrogen maleate(Sandoz, Switzerland) and indalpine (Pharmuk Lab, France).

Statistics All data were analysed using the $\bar{x} \pm SD$. The latencies obtained after the drug were compared with controls by two-way analysis of variance followed by Duncan's new multiple range test. The significance level was taken where $P<0.05$ (two-tailed).

RESULTS

Tramadol was injected intrathecally at 100, 500 and 1000 μg. Results presented

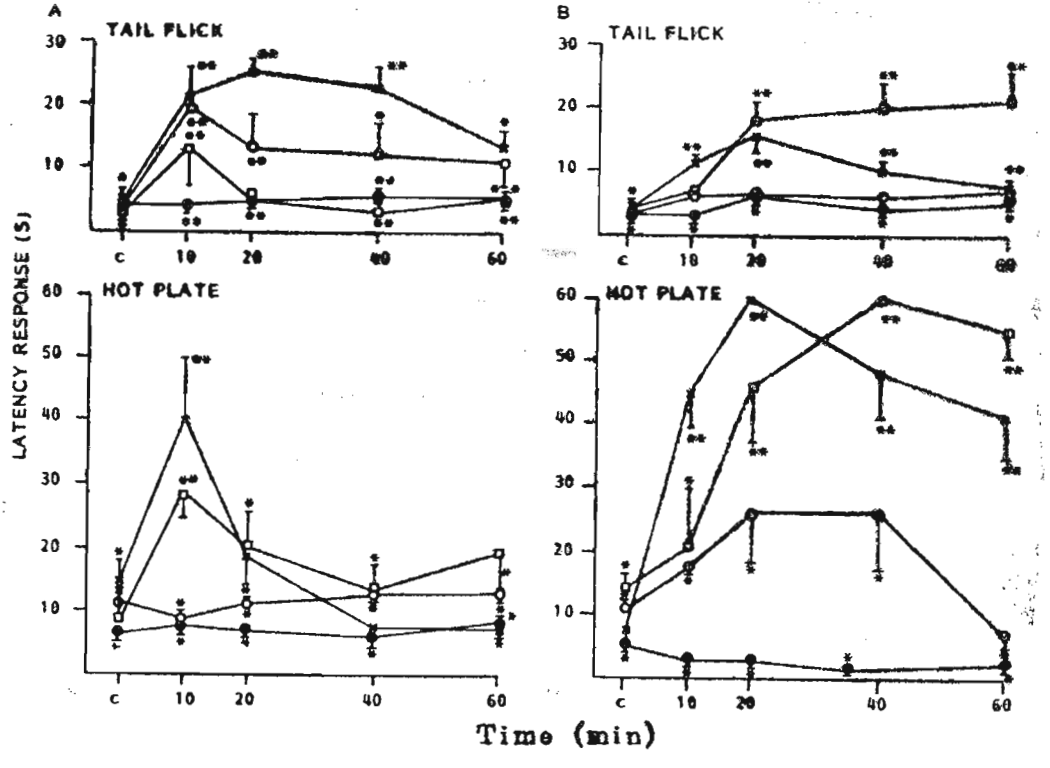

Fig 1. Intrathecal (A) and subcutaneous (B) effects of tramadol on latencies in hot-plate and tail-flick tests. In (A), intrathecal (○) saline, (●) 100, (×) 500, (□) 1000 μg tramadol. In (B), subcutaneous (○) saline, (●) 25, (×) 50, (□) 75 mg/kg tramadol. n=5, $\bar{x} \pm SD$. *$P>0.05$, **$P<0.05$.

Tab 1. Effects of ketanserin and methysergide (sc) pretreatment on the effect of tramadol (sc) on tail flick (T) and hot plate (H) latencies in the rats. n = 5, $\bar{x} \pm SD$ *$P>0.05$.

Drug (mg/kg)		Reaction time (s)				
		Control	10 min	20 min	40 min	60 min
Saline 1 ml	T	3.7±1.1	2.5±0.5*	2.5±0.5*	2.5±0.9*	2.6±0.6*
	H	5.7±2.6	4.4±2.3*	2.5±0.8*	3.2±1.8*	3.2±2.5*
Ketanserin 5	T	3.3±0.4	3.1±0.9*	3.0±0.9*	3.3±0.9*	3.0±0.7*
	H	7.6±1.6	14.1±12.8*	4.0±1.1*	21.2±17.9*	22.0±21.9*
Ketanserin 5 + Tramadol 50	T	3.8±0.7	6.4±3.8*	9.0±10.3*	9.3±11.6*	12.0±10.2*
	H	16.1±1.8	18.9±8.1*	27.8±22.4*	33.5±19.5*	16.2±4.0*
Methysergide 5	T	3.1±0.5	2.5±0.5*	2.5±0.5*	2.4±0.4*	3.1±0.6*
	H	3.1±2.4	1.5±0.8*	1.7±1.2*	2.2±0.8*	2.1±1.3*
Methysergide 5 + Tramadol 50	T	2.8±0.5	5.4±1.3*	8.3±12.1*	8.2±11.0*	3.7±0.7*
	H	11.9±3.6	31.1±21.0*	20.3±23.0*	24.0±21.5*	22.9±21.2*

Tab 2. Effects of phentolamine and indalpine (sc) pretreatments on the effect of tramadol on tail flick (T) and hot plate (H) latencies in the rats. n = 5, $\bar{x} \pm SD$. *$P>0.05$, **$P<0.05$ *vs* control.

Drug (mg/kg)		Reaction time (s)				
		Control	10 min	20 min	40 min	60 min
Saline 1 ml	T	2.1±0.5	2.7±0.8*	3.4±1.6*	3.3±1.3*	2.2±0.8*
	H	5.5±1.9	2.7±1.1*	2.6±2.5*	1.8±0.8*	2.4±2.1*
Phentolamine 5	T	4.5±0.8	4.3±0.7*	4.3±1.5*	3.4±1.3*	3.8±1.2*
	H	6.3±2.4	6.5±5.2*	7.3±4.8*	4.2±2.8*	9.0±5.4*
Phentolamine 5 + Tramadol 50	T	3.8±0.5	4.0±1.8*	5.7±1.8**	4.4±1.1*	3.1±1.3*
	H	7.2±1.6	33.1±13.2*	26.0±11.6**	30.1±17.2**	32.4±21.2**
Indalpine + Tramadol 25	T	3.1±0.9	5.5±2.2**	5.6±1.6**	10.1±5.1**	10.3±6.0**
	H	6.8±1.8	9.1±5.4*	6.2±1.8*	9.0±2.9*	8.0±3.4*

in Fig 1-A show that tramadol had a short lasting effect on tail flick and hot plate reaction times, in comparison to the saline control.

Tramadol was injected sc at 3 dose levels (25, 50 and 75 mg/kg). Tramadol did not produce any analgesic effect at a dose of 25 mg/kg, but at higher doses (50 and 75 mg/kg) a significant effect on latencies was manifested by an increase in reaction time in both the tail flick and hot plate tests (Fig 1-B). Neither ketanserin (5 mg/kg sc) nor saline had any effect on tail flick and hot plate latencies (Tab 1), but ketanserin antagonized the effect of tramadol (50 mg/kg sc) (Tab 1). However ketanserin did not antagonize the effect of sufentanil (5 μg/kg sc). Methysergide (5 mg/kg sc) also antagonized the effect of tramadol (50 mg/kg) as seen from the reaction time in the tail flick and hot plate tests (Tab 1).

Prior administration of phentolamine (5 mg/kg sc) had an antagonizing effect on tramadol in the tail flick latency tests but not in the hot plate tests. Pretreatment with 5-HT reuptake blocker indalpine (5 mg/kg sc) potentiated the effect of tramadol on latencies in the tail flick test (Tab 2).

Physostigmine (25 μg/kg sc) did not influence the reaction time in either the tail flick or hot plate tests. However pretreatment with physostigmine (25 μg/kg)

Tab 3. Effects of physostigmine and naloxone (sc) pretreatment on the effect of tramadol on tail flick (T) and hot plate (H) latencies in the rats. n = 5, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$ *vs* control.

Drug (mg/kg)		Reaction time (s)				
		Control	10 min	20 min	40 min	60 min
Saline 1 ml	T	2.9±0.9	2.8±1.5*	3.3±1.7*	2.3±0.4*	2.6±1.0*
	H	4.6±1.2	2.9±0.9*	2.4±1.5*	2.5±0.8*	2.7±0.4*
Physostigmine 0.025	T	3.1±0.6	4.1±0.9*	5.1±1.6**	4.0±1.6*	3.2±2.0*
	H	11.1±2.9	5.8±2.2*	6.5±3.4*	5.6±5.1*	6.9±2.9*
Physostigmine 0.025	T	4.2±1.1	11.8±10.5*	10.3±11.0*	10.5±8.3*	10.7±11.0*
+ Tramadol 25	H	6.1±1.3	14.8±2.9**	21.5±12.1**	52.5±16.3**	34.1±14.8*
Naloxone 2.0	T	4.5±0.9	5.6±3.1*	5.5±2.7*	5.4±2.9*	3.4±1.1*
+ Tramadol 50	H	8.2±3.8	12.2±3.6*	10.9±4.0*	13.1±5.1*	11.7±6.5*

potentiated the effect of tramadol in the hot plate latency test but not in the tail flick test. Conversely pretreatment with naloxone (2 mg/kg sc) antagonized the effect of tramadol (50 mg/kg sc) in both tail flick and hot plate tests (Tab 3).

DISCUSSION

It is generally accepted that all morphinomimetic drugs have an active effect as seen by responses in tail flick and hot plate tests[13,15]. They increase reaction time in these tests when administered intrathecally, sc or any other route. Similarly, tramadol was found to be active when administered sc and intrathecally. However, with intrathecal administration, tramadol has only a short-lasting effect (20 min) which may be due to rapid removal from the subarachnoid space.

Since it is quite effective via the sc route, it seems to have a prominent effect on the supraspinal structures. Nevertheless, to confirm this effect, further work is needed to establish the central site of action of tramadol.

There is considerable evidence in the literature[10] that the serotonergic system plays a modulating role in analgesia. Serotonin is a neurohumor in the central nervous system and its precursor and metabolites are also found there. It has been shown that serotonin plays a modulating role in the analgesic effect of morphine: morphine releases serotonin in the spinal cord and serotonin antagonists reduce the antinociceptive effect of morphine[14]. Furthermore serotonin administered intrathecally produces analgesia. In order to observe the role played by the serotonergic system on the working of tramadol, we tested the effect of serotonin antagonists (methysergide and ketanserin) on the analgesic effect of tramadol. This study demonstrated that prior administration of methysergide and ketanserin antagonised the analgesic effect of tramadol in both tail flick and hot plate tests. This was further substantiated by the fact that tramadol blocks the uptake of serotonin and noradrenaline[8], suggesting that the serotonergic system is involved in the production of analgesia by tramadol. This was confirmed by the fact that indalpine (LM 5008) which blocks serotonin uptake entirely potentiated the analgesic effect of tramadol, although this effect was only observed in the tail flick test. Apart from serotonin, the noradrenergic system is also implicated in the morphine antinociceptive effect. Furthermore, the analgesic effect of morphine is antagonised by alpha adrenergic blocking agent-phentolamine[15]. In the present study, phentolamine antagonized the analgesic effect of tramadol in the tail flick but not in the hot plate tests. The tail flick is a spinal motor reflex

which is easily antagonized. The reactions measured in the hot plate test, however, are controlled by the central nervous system and perhaps a higher dose of phentolamine is needed to antagonize this effect. Another possibility is that the noradrenergic system only works at the spinal level in producing the analgesia induced by tramadol.

Evidence for the role played by the cholinergic system in analgesia has been provided by many workers[6,3]. We used only a very low dose of physostigmine because at higher doses it has an analgesic effect itself[6]. We observed at a low dose that physostigmine potentiated the analgesic effect of tramadol, although the effect was only observed in the hot plate and not in the tail flick tests. This is probably a central effect. Thus part of the analgesic effect of tramadol is through the cholinergic system.

In conclusion, the antinociceptive effect of tramadol is due to the effect through opiate receptors while serotonergic, noradrenergic and cholinergic systems probably plays a modulating role.

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