Influence of flupirtine, a novel nonopioid analgesic agent on somatosensory evoked potentials in rats

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Abstract. The effect of flupirtine, a novel nonopioid analgesic, on somatosensory evoked potentials (SEP) was investigated in anesthetized rats. Primary somatosensory potentials were evoked in the cerebral cortex by stimulation of the skin of the whiskery part of the face. Flupirtine injected i.p. dose-dependently prolonged the latency and reduced the amplitude of SEP with ID_{50}-values of 5.4 mg/kg (2.6–9.3 mg/kg) and 7.9 mg/kg (3.9–13.8 mg/kg), respectively. This effect of flupirtine (10 mg/kg, i.p.) on the latency and the amplitude of SEP, did not change when naloxone (1 mg/kg, i.p.) was given before flupirtine. The results indicate that the analgesic flupirtine decreases the primary somatosensory evoked potential by diminishing the excitability of cortical neurons. Opioid mechanisms are not involved.
Key words. Flupirtine; morphine; naloxone; somatosensory evoked potential; rat.

Flupirtine (ethyl-N-2-amino-6-(4-fluoro-phenylmethyl-amino)pyridine-3yl maleate), a novel nonopioid analgesic has been shown to inhibit nociceptive responses induced by chemical, thermal, mechanical and electrical stimuli in rodents[1]. Earlier results[2,3] suggested that flupirtine produces its analgesic effect by action at the spinal and the supraspinal level. It has also been shown that the mode of antinociceptive action of flupirtine differs from that of opioids, because flupirtine has no affinity for opiate receptors[3] and its analgesic activity cannot be abolished by naloxone[1]. Furthermore, flupirtine has a dose-dependent antinociceptive action after intrathekal or microinjection in the PAG. Flupirtine also depresses the response of ascending axons to afferent C fiber stimulation (sural nerve) after i.v. injection into rats with intact spinal cord or intrathekal injection into decerebrated spinal rats[4].

In addition flupirtine dose-dependently reduces nociceptive activity in the thalamus in rats[3,5]. These results show that flupirtine produces analgesia by spinal inhibition of nociceptive impulse transmission from afferent nerve fibres to neurons sending their axons to the brain, and by supraspinal inhibition of nociceptive impulse transmission to the thalamus.

Administration of flupirtine results in an intensification of almost all frequency bands in the EEG of the rat, similar to that induced by clonidine[6]. It has been demonstrated that the antinociceptive activity of flupirtine can be diminished by alpha adrenoceptor antagonists[7]. More detailed studies have shown that flupirtine is not an $alpha_2$-adrenoceptor agonist. However, recent results clearly indicate that the descending noradrenergic pain-modulating system is involved in the antinociceptive action of flupirtine.

Somatosensory evoked potentials (SEP) are enhanced by morphine in the rat[8]. In order to find further differences between the modes of action of flupirtine and morphine, we investigated the effect of flupirtine on SEP in rats.

Materials and methods

The experiments were carried out on male Wistar rats (200–220 g b.wt) (LATI, Hungary). The animals were maintained under standard environmental conditions (room temperature: 21–22 °C; relative humidity: 55–60%; light-dark-rhythm: 12/12 h). They had free access to standard pellet food and drinking water, apart from the 16 h before the experiments when they were deprived of food.

The rats received an i.p. injection of chloralose (110 mg/kg) and urethane (770 mg/kg) to induce and maintain anesthesia for surgery and the experiment. The animals were fixed in a stereotactic device and a hole was drilled into the skull for introduction of a microelectrode (tip diameter 1 µm; resistance 30 ohm) over the parietal lobe of the brain. For recording activity from the primary somatosensory cortex, the electrode was positioned according to rat brain atlas[9]. For stimulation, two platinum wire electrodes were placed 2 mm apart on the skin of the whiskery part of the face. Rectangular supramaximal impulses (0.2 ms, 2 V, 0.5 Hz; Hugo Sachs Electronic, Hugstetten, FRG) were employed for stimulation.

Potentials recorded from the cortex were passed through a window discriminator (Cambridge Electronic Ltd, Cambridge, UK) and evaluated with a personal computer (Tandon, Frankfurt, FRG) using an averaging program (Cambridge Electronic Ltd, Cambridge, UK). Latencies and peak amplitudes were determined from the average of 25 SEP each. The basal value was determined 10 min before drug application. Flupirtine was administered in a single dose to each rat, alone or in combination with naloxone. Statistical differences were established by using Student's t-test for paired samples. ED_{50}-values were calculated by ANOVA.

The following drugs were used: flupirtine (ASTA Medica, Frankfurt, FRG), naloxone, chloralose and urethane (all from Sigma, Deisenhofen, FRG). Compounds were dissolved in physiological saline.

Table 1. Influence of flupirtine on the latency (L) and amplitude (A) of somatosensory evoked potentials in anesthetized rats (mean values ± SEM)

	n	Basal value L (ms)	Basal value A (μV)	30 min L (ms)	30 min A (μV)	60 min L (ms)	60 min A (μV)	90 min L (ms)	90 min A (μV)
Saline control	6	3.6 ± 0.19	344 ± 20.4	3.1 ± 0.11	310 ± 19.1	3.5 ± 0.13	331 ± 14.6	3.2 ± 0.17	328 ± 25.6
Flupirtine (mg/kg, i.p.)									
2.5	6	3.8 ± 0.16	353 ± 18.7	4.4 ± 0.15*#	270 ± 17.9*	4.1 ± 0.18	301 ± 21.6	3.9 ± 0.19	331 ± 26.5
5.0	6	3.0 ± 0.14	305 ± 16.9	4.6 ± 0.19**##	180 ± 20.4**##	4.8 ± 0.13**#	190 ± 17.6**##	3.6 ± 0.18*#	280 ± 25.6
10.0	6	3.1 ± 0.17	334 ± 23.6	5.1 ± 0.13**##	135 ± 17.1**##	5.0 ± 0.15**##	181 ± 16.7**##	3.9 ± 0.17*#	223 ± 22.6**##
Flupirtine (10 mg/kg, i.p.) + naloxone (1 mg/kg, i.p.)	6	3.3 ± 0.13	350 ± 21.9	5.4 ± 16**##	141 ± 17.7**##	5.1 ± 0.18**##	170 ± 16.9**##	4.1 ± 0.17*#	285 ± 24.6*#

n = number of animals; * significant difference from basal value: * $p < 0.05$, ** $p < 0.01$; # significant difference from saline control: # $p < 0.05$, ## $p < 0.01$.

Table 2. Comparison of the EEG-changes in percentages related to the control value for the same animal after i.p. administration of flupirtine in anesthetized or freely moving rats

Compound	Dose (mg/kg, i.p.)	Time after treatment (min)	Anesthetized rats EEG frequency bands Delta 1.5 – 3.5	Theta 3.7 – 7.5	Alpha 7.8 – 13.5	Beta 13.8 – 30.0	Freely moving rats EEG frequency bands Delta 1.5 – 3.5	Theta 3.7 – 7.5	Alpha 7.8 – 13.5	Beta 13.8 – 30.0 Hz
Saline 0.9%		30	+ 4 ± 3	− 6 ± 4	− 7 ± 5	+ 7 ± 4	− 3 ± 2	+ 6 ± 5	− 8 ± 6	+ 6 ± 4
Flupirtine	5	30	− 22 ± 8**	+ 24 ± 6**	+ 27 ± 8**	+ 16 ± 4**	− 16 ± 4**	+ 18 ± 6**	+ 22 ± 4**	+ 12 ± 6*

Asterisks indicate that the EEG frequency changes after administration of drug differed significantly from the baseline value for the same animal, obtained before application of drug (* $p < 0.05$; ** $p < 0.01$).

Results

In contrast to the saline-treated animal group, flupirtine prolonged the latencies of SEP and reduced the amplitudes of SEP in a dose-dependent manner (table 1). The onset of its effect was rapid (5 min after drug administration) and maintained for more than 90 min. At the time of maximum effect (30 min p.a.), ED_{50}-values of 5.4 (2.6–9.3) and 7.9 (3.9–13.8) mg/kg i.p. were calculated for prolongation of latency and reduction of amplitude, respectively. Following the maximum effect the changes in SEP induced by flupirtine slowly returned to the basal value. Full normalization was achieved 120 min after drug administration. The effect of flupirtine on SEP in the rat remained unchanged following pretreatment with naloxone (1 mg/kg i.p.) (table 1). The influence of flupirtine on the EEG of anesthetized or freely moving rats did not differ significantly (table 2).

Discussion

The present results show that flupirtine prolongs the latency and reduces the amplitude of SEP in anesthetized rats, an effect which is different from that observed after administration of morphine to rats[6]. Soto-Moyano and Hernandez[10] observed that after topical administration of 1% morphine to the primary somatosensory area of the rat, SEP activity was significantly enhanced. By contrast, flupirtine induced opposite effects in SEP, which strongly suggests that it has the ability to depress responses evoked by somatosensory afferent stimulation in which opioid mechanisms are not involved, and that its mode of analgesic action is different from that of opioids.

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