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BRIEF COMMUNICATION

Effects of the Neuroleptanalgesic Fentanyl-Fluanisone (Hypnorm) on Spike-Wave Discharges in Epileptic Rats

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INOUE, M., N. ATES, J. M. H. VOSSEN AND A. M. L. COENEN. *Effects of the neuroleptanalgesic fentanyl-fluanisone (Hypnorm) on spike-wave discharges in epileptic rats.* PHARMACOL BIOCHEM BEHAV **48**(2) 547–551, 1994.—Effects of fentanyl–fluanisone (Hypnorm), a combination often used as a neuroleptanalgesic and anaesthetic, were investigated on spike-wave discharges of epileptic WAG/Rij rats. Fentanyl–fluanisone has stimulating effects on the amount of spike-wave discharges, but not in a dose-dependent manner. A low dose of 0.01 mg/kg fentanyl with 0.5 mg/kg fluanisone causes a large increase in epileptic activity. This effect is larger than with a middle dose of 0.1 mg/kg fentanyl and 5 mg/kg fluanisone and much larger than with a high dose of 0.2 mg/kg fentanyl with 10 mg/kg fluanisone. The last two doses cause a prolonged anaesthetic state in rats. The frequency of the spikes in the spike-wave discharges was decreased by the mixture of fentanyl–fluanisone; this decrease was dose-dependent.

Administration of fentanyl alone in the same doses as in the combination abolishes spike-wave activity during the anaesthetic phase but causes a moderate increase after this phase. It also causes a lowering in spike frequency, which is of the same order as with fentanyl–fluanisone. The mechanisms of action of fentanyl are not completely clear, but the opposite effects of μ- and κ-opioid receptors on spike-wave discharges may play a role in this biphasic effect. Fluanisone alone in the same doses as in the mixture induces a large dose-dependent increase in spike-wave activity, with only a small effect on spike frequency. This might be caused by the antagonistic action of this drug at dopamine receptors. The complexity of the stimulatory effects on spike-wave discharges caused by fentanyl–fluanisone is due to an interaction of the suppressive-stimulatory effects of fentanyl together with the facilitatory effects of fluanisone.

Fentanyl Fluanisone Hypnorm Neuroleptanalgesia Anesthesia Absence epilepsy
Spike-wave discharges Genetic epilepsy model WAG/Rij rat

A COMBINATION of fentanyl and fluanisone, better known under the trade name Hypnorm, is widely used in anaesthetic practice for the induction of neuroleptanalgesia in small laboratory animals (7). Fentanyl is a potent narcotic analgesic with properties similar to the opioid agonist morphine (8) and is often used in humans as an analgetic and in higher doses as an anaesthetic (1). Fluanisone is a putative antipsychotic with dopamine antagonistic properties, belonging to the class of the butyrophenones (14).

Both the opioidergic and dopaminergic systems in the brain modulate spontaneously occurring spike-wave discharges in seizure-prone rats (10,15). Haloperidol, which belongs to the same group of butyrophenones as fluanisone, produces a dose-dependent increase of spike-wave discharges (9,15), whereas the morphine-like drug fentanyl also leads to an increase in these paroxysms (6). Since the combination of fentanyl and fluanisone is often used in anaesthesia, it is important to know whether this drug combination also has facilitatory effects on spike-wave discharges. In the present study we tested the mixture of fentanyl–fluanisone as well as both drugs sepa-

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rately in rats of the WAG/Rij strain. All individuals of this strain spontaneously show spike-wave discharges in the cortical EEG; this strain of rats is regarded as a valid animal model for absence epilepsy (2,12,13).

METHODS

A total of 18 WAG/Rij rats of both sexes and five to eight months of age were used. Rats were housed separately and were maintained on a 12-h light–dark cycle with white lights on at 21:00 h. They had ad lib access to standard food and tap water. Two gilded screws which served as electrodes were permanently implanted under pentobarbital anaesthesia (60 mg/kg) and were positioned on the dura, the first on the frontal cortex (coordinates with skull surface flat and bregma 0-0: A 2.0, L 3.5) and the second on the parietal cortex (A −6.0, L 4.0). A ground electrode was placed over the cerebellum. All electrodes were connected to a receptacle fixed to the skull surface. Following surgery, subjects were allowed to recover for 10 days and were habituated to the experimental conditions. The EEG was registered on a polygraph with filter settings between 1 and 70 Hz and a paper speed of 1 cm/s. In all experiments the EEG was recorded during a 1-h baseline period just before drug administration and during six periods of 30 min after injection. For each rat the amount of spike-wave discharges was determined in the baseline and in the six postinjection periods. This amount was expressed as a percentage of time which was occupied by spike-wave discharges. Criteria for measuring and identifying spike-wave discharges are described elsewhere (12). The frequency of the spikes in the spike-wave discharges was measured in the first spike-wave discharge of each time period.

Fentanyl–fluanisone was tested in six rats. The compound contained 0.2 mg fentanyl and 10 mg fluanisone per 1 ml solvent (Hypnorm, Janssen Pharmaceutics, Beerse, Belgium). Fentanyl–fluanisone was intramuscularly administered in doses of 0.05 ml/kg (0.01 mg/kg fentanyl–0.5 mg/kg fluanisone), 0.5 ml/kg (0.1 mg/kg fentanyl–5 mg/kg fluanisone), and 1.0 ml/kg (0.2 mg/kg fentanyl–10 mg/kg fluanisone). Experimental days were separated by six or seven days and all rats were used four times in a counterbalanced design, for a saline control recording and for the three drug doses.

Twelve rats were used for the separate tests of fentanyl and fluanisone. Fentanyl was dissolved in saline in a concentration of 0.2 mg/ml, which is comparable to that of the fentanyl–fluanisone combination. Doses of 0.1 mg/kg (0.5 ml/kg) and 0.2 mg/kg (1 ml/kg) were tested. Fluanisone was also dissolved in saline with some drops of lactate and intramuscularly injected in doses of 0.5, 5, and 10 mg/kg. Each group consisted of six rats in a counterbalanced design. Analysis of variance (ANOVA) and Fisher post hoc tests were used for statistical analyses.

RESULTS

Effects of the fentanyl–fluanisone compound on the amount of spike-wave discharges are illustrated in Fig. 1a. In the baseline period all rats showed spike-wave discharges on an average of 7.2% of total time. Following injection of the compound, the amount of spike-wave activity significantly increased for all doses during the first 30 min as compared with saline (ANOVA, $p < 0.001$). This increase was not dose-related, but the largest increase was found with the lowest dose (0.01 mg/kg fentanyl–0.5 mg/kg fluanisone), followed by the middle dose of 0.1 mg/kg fentanyl–5 mg/kg fluanisone and thereafter followed by the highest dose of 0.2 mg/kg fentanyl–10 mg/kg fluanisone (Fisher, $p < 0.05$). A decrease of the frequency of the spikes in the discharges was observed in all groups (ANOVA, $p < 0.01$) (Fig. 2a). With the lowest dose the frequency decreased slightly from 9 Hz to 7 Hz, with the middle dose to 6 Hz and the highest dose to 5 Hz (Fisher, $p < 0.05$).

With the lowest dose the background activity in the EEG was similar to that of wakefulness and sometimes to that of light slow-wave sleep. Rats were immobilized but not anaesthetized during the first 30 min. With the middle dose, interictal EEG activity resembled that of slow-wave sleep, and an anaesthetic state was seen until 60–90 min after injection. With the highest dose the background activity was similar to that of deep slow-wave sleep and the anaesthetic state lasted until the end of the recording period.

Fentanyl alone also affected the amount of spike-wave discharges (ANOVA, $p < 0.05$) (Fig. 1b). With a dose of 0.1 mg/kg fentanyl, rats were in an anaesthetic state from 30 to 60 min after injection and showed almost no spike-wave discharges during that period. After that time, spike-wave discharges appeared again, but this increase did not significantly differ from the saline group. With a dose of 0.2 mg/kg fentanyl, the duration of the anaesthetic state was prolonged to 80 min, and during that time rats also showed almost no paroxysms. With this dose the delayed increase of discharges after the anaesthetic state reached significance with saline at 120–150 min and at 150–180 min after injection (Fisher, $p < 0.05$). A lowered spike frequency in the spike-wave discharges was also observed with both doses (ANOVA, $p < 0.001$) (Fig. 2b). The frequency was diminished to 6 Hz with 0.1 mg/kg and to 5 Hz with 0.2 mg/kg fentanyl (Fisher, $p < 0.05$). The time course of the changes in spike frequency was in general concurrent with the length of the anaesthetic state.

Effects of fluanisone on spike-wave discharges are presented in Fig. 1c. Following administration of 0.5, 5, and 10 mg/kg fluanisone, the total amount of spike-wave discharges increased significantly and dose-dependently in all periods compared to saline (ANOVA, $p < .001$). After all three doses of fluanisone, animals were most of the time in an immobilized state but not anaesthetized. There were small but significant changes in the spike frequency of the discharges (ANOVA, $p < 0.05$) (Fig. 2c). Fluanisone in a dose of 0.5 mg/kg did not change this frequency, but with a dose of 5 and 10 mg/kg spike frequency was lowered to 7.5 and 6.5 Hz, respectively (Fisher, $p < 0.05$).

DISCUSSION

The present study shows that the combination of fentanyl and fluanisone (Hypnorm) has facilitatory effects on the occurrence of spike-wave activity in rats, but with a complex time course and not in a dose-related way. Administration of fentanyl alone shows that this drug initially abolishes spike-wave activity almost completely, while this decrease is followed by a prolonged increase. The second drug, fluanisone, produces a large increment in spike-wave activity. Therefore, we suggest that the main stimulatory effects on spike-wave activity of fentanyl–fluanisone are caused by the effects of fluanisone and that the inhibitory phase seen with the higher doses of fentanyl–fluanisone is due to the initial inhibitory effects produced by fentanyl. When this inhibitory effect of fentanyl diminishes and changes into a facilitating one, an enhancement of spike-wave activity becomes still more prominent. On the other hand, a dose-dependent decrease in the frequency of the spikes in the spike-wave discharge is found

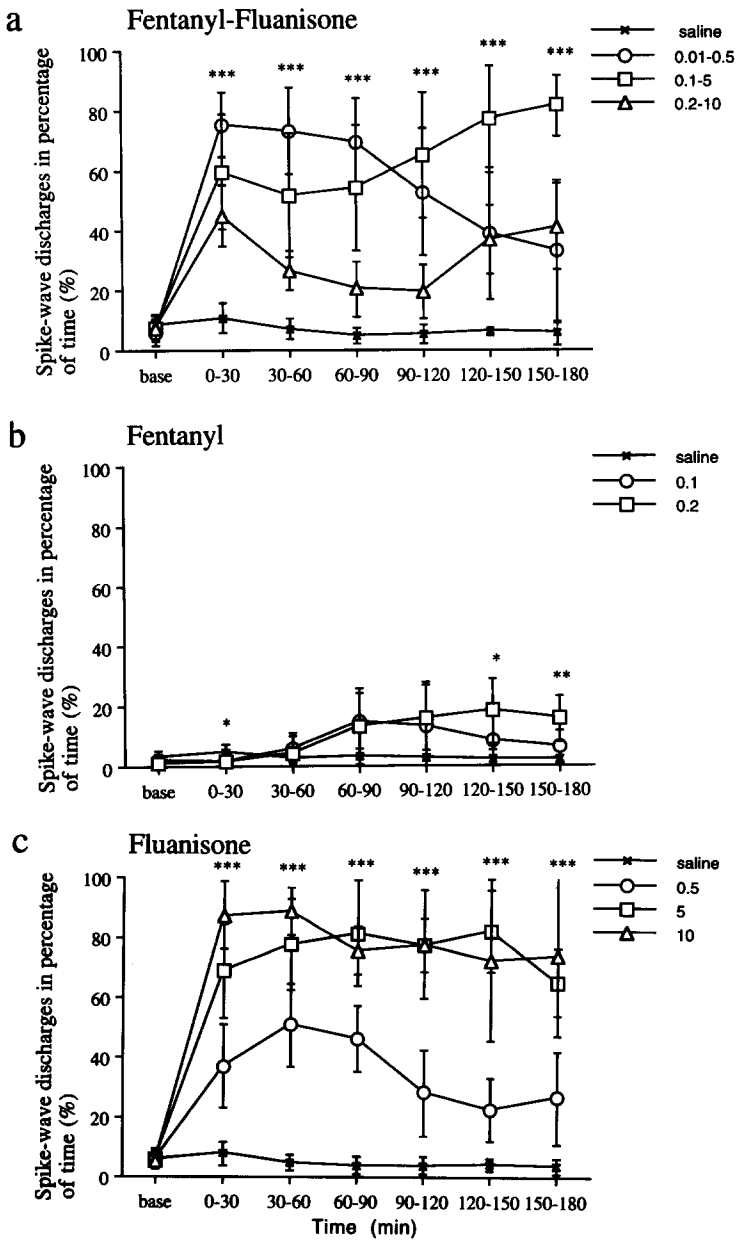

FIG. 1. Effects of (a) fentanyl-fluanisone, (b) fentanyl, and (c) fluanisone on spike-wave discharges of WAG/Rij rats. The amount of spike-wave discharges with SD is expressed as percentage of time of their occurrence in the 30-min periods indicated on the X axis ("base" is the baseline period and 0 is the time of injection). Concentrations of the drugs are indicated at right in mg/kg, and statistics are explained in the Results section. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

with fentanyl-fluanisone, whereby the main action can be attributed to the effects of fentanyl.

The inhibitory effects of fentanyl both on the amount of spike-wave discharges and on the spike frequency are fairly well correlated with the duration of the anaesthetic period. The anaesthetic period is longer when anaesthesia is induced by fentanyl-fluanisone than with fentanyl alone, for the reason that fluanisone potentiates the actions provided by fentanyl (7). The time course of the anaesthetic state induced by fentanyl-fluanisone also corresponds to that phase in which the amount of spike-wave activity is low and in which the spike frequency is lowered. This may suggest that the mechanisms causing anaesthesia are associated with the mechanisms producing the inhibition of spike-wave activity. However, the anaesthetic etomidate, which heavily facilitates spike-wave activity, jeopardizes this view (5). It is therefore more likely that

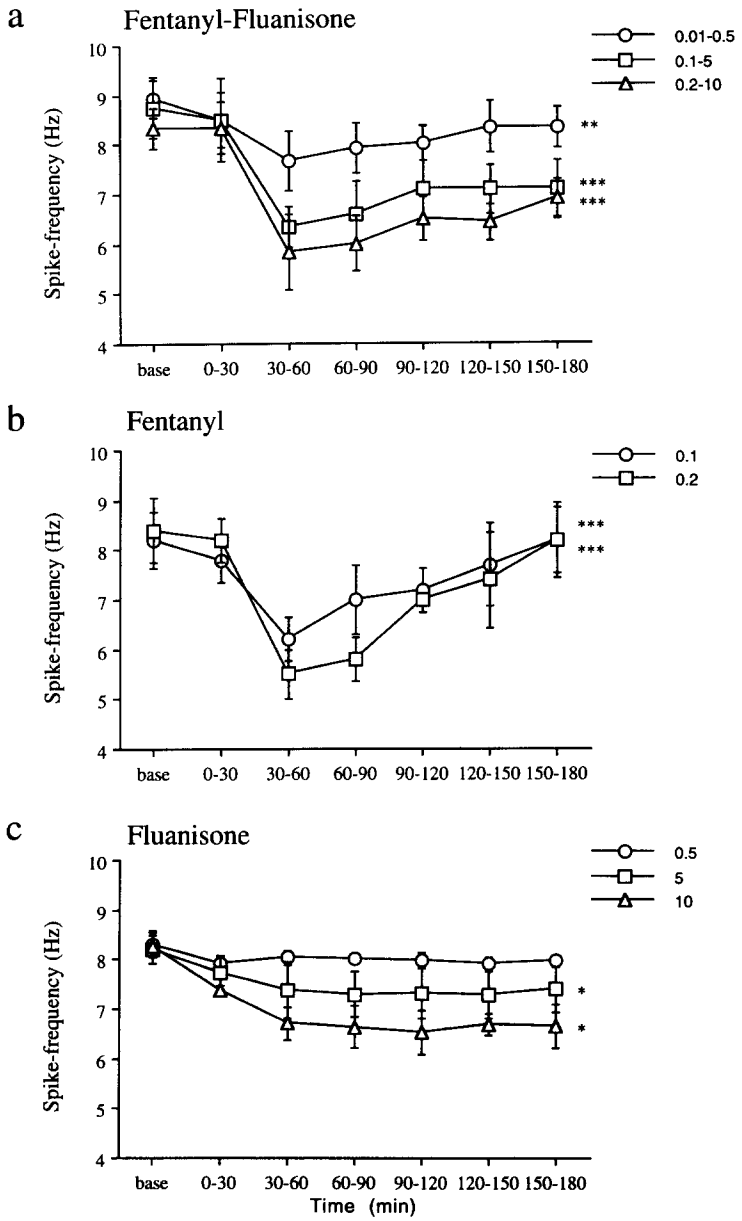

FIG. 2. Effects of (a) fentanyl-fluanisone, (b) fentanyl, and (c) fluanisone on the frequency of spikes with SD in the spike-wave discharges of WAG/Rij rats. This frequency was determined in the first discharge of every period of 30 min indicated on the X axis and in the 30-min baseline period ("base" is the baseline period and 0 is the time of injection). Concentrations of the drugs are indicated at right in mg/kg, and statistics are explained in the Results section. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

the modulation of discharges is caused by direct effects of the drugs itself, interacting with neurotransmitter systems.

The mechanisms of action of the opioid agonist fentanyl are not fully understood, but recently it was reported that fentanyl in low concentrations produces an increase in paroxysms (6). This is in line with the present findings, if we take the overall epileptic activity over the complete recording period. Furthermore, it is found that the opioid agonist DAMGO, which specifically acts at μ-receptors, potentiates the occurrence of spike-wave discharges (10). It is known that fentanyl also more or less specifically acts at μ-receptors, and the increase of spike-wave discharges by fentanyl in low doses could be due to the effects on the μ-receptor. Besides a high affinity of fentanyl for the μ-receptor, this drug also has a low affinity for κ-receptors (11). Since κ-agonists such as U50.488 inhibit spike-wave discharges (10), it is possible that in higher doses κ-induced inhibition overrules μ-induced excitation.

Fluanisone is a dopamine receptor antagonist comparable

to haloperidol (14). It is already reported that these antagonists have a strong enhancing effect on the amount of spike-wave activity (9,15). Furthermore, two selection lines of Wistar rats characterized by opposite dopaminergic activities also prominently differ in the number of spike-wave discharges (3). With respect to the opioids, Di Chiara and Imperato (4) described opposite effects of μ- and κ-opioid agonists on dopamine release in the brain of rats. In all, the present finding confirms the conclusion by Warter et al. (15) that the dopaminergic system participates in the control of absence seizures. Nevertheless, the detailed nature of this participation has still to be elucidated.

In short, the present article shows that the often-used drug combination fentanyl–fluanisone has, in addition to anaesthetic and analgesic properties, seizure-facilitating properties due to its modulation of the dopaminergic system. This (pro)-convulsant action necessitates a careful use of this neuroleptanalgetic drug combination.

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REFERENCES

1. Bailey, P. L.; Stanley, T. H. Narcotic intravenous anaesthetics. In: Miller, R. D., ed. Anesthesia, 3rd ed. New York: Churchill Livingstone; 1990:281–367.
2. Coenen, A. M. L.; Drinkenburg, W. H. I. M.; Inoue, M.; Van Luijtelaar, E. L. J. M. Genetic models of absence epilepsy, with emphasis on the WAG/Rij strain of rats. Epilepsy Res. 12:75–86; 1992.
3. Cools, A. R.; Peeters, B. W. M. M. Differences in spike-wave discharges in two rat selection lines characterized by opposite dopaminergic activities. Neurosci. Lett. 134:253–256; 1992.
4. Di Chiara, G.; Imperato, A. Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. J. Pharmacol. Exp. Ther. 244:1067–1079; 1988.
5. Duysens, J.; Inoue, M.; Van Luijtelaar, E. L. J. M.; Dirksen, R.; Coenen, A. M. L. Facilitation of spike-wave activity by the hypnotic etomidate in a rat model for absence epilepsy. Int. J. Neurosci. 27:213–217; 1991.
6. Frey, H.-H.; Voits, M. Effect of psychotropic agents on a model of absence epilepsy in rats. Neuropharmacology 30:651–656; 1991.
7. Green, C. J. Neuroleptanalgesic drug combinations in the anaesthetic management of small laboratory animals. Lab. Anim. 9: 161–178; 1975.
8. Janssen, P. A. J.; Niemegers, C. J. E.; Dony, J. G. H. The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. Arzneimittelforschung 13:502–507; 1963.
9. Kleinlogel, H. Spontaneous EEG paroxysms in the rat: Effect of psychotropic and alpha-adrenergic agents. Neuropsychobiology 13:206–213; 1985.
10. Lason, W.; Przewlocka, B.; Przewlocki, R.; Coenen, A.; Van Luijtelaar, G. The role of opioid mechanisms in nonconvulsive seizures in WAG/Rij rats. In: van Ree, J. M.; Mulder, A. H.; Wiegant, V. M.; van Wimersma Greidanus, T. B., eds. New leads in opioid research. Amsterdam: Excerpta Medica; 1990:350–352.
11. Maguire, P.; Tsai, N.; Kamal, J.; Cometta-Morini, C.; Upton, C.; Loew, G. Pharmacological profiles of fentanyl analogs at mu, delta and kappa opiate receptors. Eur. J. Pharmacol. 213:219–225; 1992.
12. Van Luijtelaar, E. L. J. M.; Coenen, A. M. L. Two types of electrical paroxysms in an inbred strain of rats. Neurosci. Lett. 70:393–397; 1986.
13. Van Luijtelaar, E. L. J. M.; Coenen, A. M. L. The WAG/Rij model for generalised absence seizures. In: Manelis, E. B.; Loeber, J. N.; Dreifuss, F. E., eds. Advances in epileptology, vol. 17. New York: Raven Press; 1989:78–83.
14. Van Wijngaarden, I.; Kruss, C. G.; Van Hes, R.; Van der-Heyden, J. A. M.; Tulp, T. M. 2-Phenylpyrroles as confirmationally restricted benzamide analogues. A new class of potential antipsychotics. J. Med. Chem. 30:2099–2104; 1987.
15. Warter, J. M.; Vergnes, M.; Depaulis, A.; Tranchant, C.; Rumbach, L.; Micheletti, G.; Marescaux, C. Effects of drugs affecting dopaminergic neurotransmission in rats with spontaneous petit mal-like seizures. Neuropharmacology 27:269–274; 1988.