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Comparative study of the sedative and antinociceptive effects of levomepromazine, azaperone and midazolam in laboratory animals

[*Estudo comparativo dos efeitos sedativos e antinociceptivos de levomepromazina, azaperone e midazolam em animais de laboratório*]

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ABSTRACT

The sedative and antinociceptive effects of levomepromazine, azaperone and midazolam were studied in rats and mice using three behavior evaluation methods. Both exploratory behavior and spontaneous locomotor activity were significantly diminished in a spontaneous locomotor activity test in open field when using levomepromazine and azaperone. However, the azaperone effects were short lived in comparison to levomepromazine effects. Midazolam caused reduction in exploratory activity with no effect in spontaneous locomotion. When assessing the antinociceptive effect in the tail flick reflex latency test after infliction of a pain stimulus in rats, tested drugs did not show any antinociceptive effect. The drugs studied were able to abolish the writhing reflex in mice when compared to control. Levomepromazine, azaperone and midazolam, at the doses were able to inhibit the exploratory behavior in rats, proving their sedative effect. Regarding the antinociceptive effects for visceral pain, these drugs were able to block contortions in mice.

Keywords: sedation, nociception, levomepromazine, azaperone, midazolam

RESUMO

Os efeitos sedativos e antinociceptivos da levomepromazina, azaperone e midazolam foram avaliados utilizando-se três testes de comportamento em ratos e camundongos. No teste da atividade locomotora espontânea em campo aberto observou-se que tanto o comportamento exploratório como a atividade locomotora espontânea foram significativamente diminuídos quando se utilizou levomepromazina e azaperone. O efeito causado pelo azaperone foi menos prolongado quando comparado ao da levomepromazina. O midazolam causou diminuição do comportamento exploratório sem alterar a atividade locomotora espontânea. Quando se avaliou o efeito antinociceptivo por meio da latência para o reflexo da retirada da cauda em ratos após estímulo doloroso, as drogas não apresentaram nenhum efeito antinociceptivo observável. No teste das contorções em camundongos, os fármacos foram capazes de abolir as contorções quando comparados ao efeito do grupo-controle. Levomepromazina, azaperone e midazolam nas doses utilizadas foram capazes de inibir o comportamento exploratório de ratos, comprovando seus efeitos sedativos. Com relação aos efeitos antinociceptivos para dor visceral, eles foram capazes de inibir as contorções.

Palavras-chave: sedação, nocicepção, levomepromazina, azaperone, midazolam

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INTRODUCTION

Various tranquilizers are used as sedatives in veterinary medicine, among them, there are major and minor tranquilizers according to the duration of the effect. Major tranquilizers are used in animals mainly as pre-anesthetic medication, potentiators of analgesia (neuroleptoanalgesia) and antiemetics (Spinosa et al., 1999), while in humans levomepromazine is used in the treatment of psychiatric disorders and as an analgesic as well (Hals and Dahl, 1995). Patt et al. (1994) reported that neuroleptic drugs, specifically phenothiazines, have an important role in the control of behavioral symptoms such as agitation, delirium and nausea.

Phenothiazines produce depression of the central nervous system (CNS), sedation, muscle relaxation and reduction in spontaneous activity (Spinosa et al., 1999). When utilized as pre-anesthetics, they can reduce considerably the dose of the anesthetic agent to be employed, thus making patient's recuperation more rapid (Thurmon et al., 1996).

Another drug from this group of major tranquilizers is the butyrophenone azaperone, and its role as a pre-anesthetic has been reported by Olson and Renchko (1988). These authors observed that this drug administered alone produced sedation in mice, without, meanwhile, producing analgesia. However, sedation in rats was accompanied by elimination of the avoidance reflex to a pain stimulus, suggesting the occurrence of a sedative and possibly of an antinociceptive effect in this species.

Midazolam is a minor tranquilizer, short-acting, water-soluble benzodiazepine. It is utilized worldwide as a hypnotic and anxiolytic. This drug has been reported to have antinociceptive activity in behavior models of acute pain when administered spinally (Goodchild and Serrao, 1987; Crawford et al., 1993).

Kissin et al. (1992) studied locomotor activity after recuperation from hypnosis in rats. Two groups of drugs were used in this study. The first was only the benzodiazepine midazolam (20mg/kg IV) and the second a combination of midazolam (4mg/kg IV) and morphine (1.3mg/kg IV). The difference found between the two groups in locomotor activity after recuperation from hypnosis was very pronounced, demonstrating that sedation, after recuperation, is lessened if a combination of morphine and midazolam is used. This finding was explained by a synergistic interaction of the drugs, allowing a significant reduction of the benzodiazepine dose. In contrast, Petersen et al. (1996) demonstrated that the interaction between midazolam and morphine increases the sedative and hypnotic actions of these drugs due to the synergism between them.

The aim of this study was to compare the sedative drugs levomepromazine, azaperone and midazolam, regarding their sedative and antinociceptive effects.

MATERIALS AND METHODS

Adult Wistar rats (n=180) and adult Swiss mice weighing approximately 250 and 30g, respectively, were obtained from Central Animal Facility of UNESP, Botucatu Campus, Brazil, and used in this study. These animals (Table 1) received drugs or saline (control) only once and were then sacrificed.

Table 1. Number of animals used according to treatment group

Animal \ Drug		Saline	Levomepromazine (10mg/kg i.p.)	Azaperone (3,5mg/kg i.p.)	Midazolam (8mg/kg i.p.)
Rat	SLA	30	10	10	10
	TFRL	30	10	10	10
Mice	MWT	30	10	10	10

SLA= spontaneous locomotor activity; TFRL= tail flick reflex latency; MWT= mice writhing test.

The sedative and antinociceptive effects were determined for the following drugs and doses: levomepromazine[1] (10.0mg/kg ip), azaperone[2] (3.50mg/kg ip) and midazolam [3] (8.0mg/kg ip), utilizing the methods of the rat spontaneous locomotor activity (SLA), tail flick reflex latency (TFRL) and mice writhing test (MWT).

Sedation was assessed by measuring locomotor activity of animals in the absence or presence of drug before and after medication. Locomotor activity was quantified utilizing the open-field method (Broadhurst, 1960). It was carried out in a circular arena made of white synthetic laminate[4] (Figure 1), 97cm in diameter and 32.5cm in height, and divided into three concentric circles which are subdivided by line segments into 22 approximately equal parts.

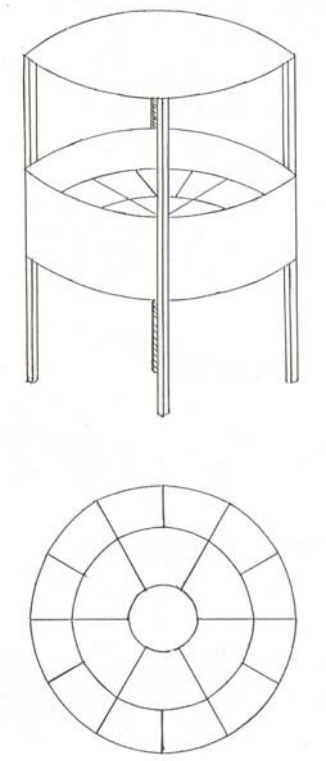

Figure 1. Squematic representation of the open field for locomotor activity evaluation, carried out in a white colored circular arena.

[1] Neozine®, Rhodia Farma Ltda – São Paulo – SP
[2] Stressnil®, Jansen Pharmaceutica – Paulinia – SP
[3] Dormonid®, Produtos Roche Químicos e Farmacêuticos S.A. - Rio - RJ
[4]Formica®

In order to measure locomotor activity, all animals were placed individually in the center of the arena and observed for 6min. The time points utilized were delineated as follows: after drug administration, 8 – 14min (T1), 22 – 28min (T2), 36 - 42min (T3).

Animals in the control and experimental groups were observed alternately always in the morning. After the observation of each rat, the arena was cleaned with 5% alcohol solution (v/v) in order to remove any odor that could influence the behavior of the next rat to be tested.

Each unit of locomotion (SLA) corresponded to the animal entering, with its four limbs, into a different division of the arena floor. Recording of the frequencies of the parameters was carried out using a manual counter, determining the total number of movements during a 6-min period.

Determination of antinociception, regarding time and dose-response for each drug was carried out with a heat projection lamp constructed by the Office of Precision at FCAV/UNESP, as described elsewhere (Kamerling et al., 1985a; Kamerling et al., 1985b).

The quick exposure of the rat's tail to the heat of a light bulb was utilized as the pain stimulus to induce the withdrawal reflex of the tail. The tail flick reflex latency (TFRL) which is defined as the time-lag between the application of heat from the hot light bulb and the withdrawal of the tail (avoidance) by the rat, was then measured. The pain stimulus was always interrupted when the time of exposure reached a maximum of 10 sec to prevent injury to the tissues.

A second lamp, not directed to the animal, was frequently utilized to confuse the animal and reduce the possibility of the occurrence of a conditioned reflex to the light, instead of the pain stimulus produced by the focalized beam of light.

The TFRL was measured immediately before and after the injection of saline (control) or drug (Table 1). The measure of latency time obtained before the injection was used to establish control values for each rat (T0). The latency times were determined at 10 (T1), 20 (T2) and 30 min (T3) after drug administration.

To perform the mice writhing test (MWT), the animals were divided into control and test groups, 10 animals per group (Table 1), which received ip saline (control groups) or drug (test groups). Ten minutes after the injection of these substances, 0.6% acetic acid was administered by the same route as the pain stimulus. After another 10min, the number of writhings was counted during a period of 20min.

Statistical evaluation was performed by analysis of variance followed by comparison of the means using Tukey's test, whereby the level of significance was established at $P<0.05$.

RESULTS AND DISCUSSION

Figure 2 shows the spontaneous locomotor activity (SLA) of rats in open field, in response to the administration of saline (control), 10 mg/kg levomepromazine, 3.5 mg/kg azaperone and 8mg/kg midazolam. SLA of the control group over time showed significant differences between the results at T1 and those obtained at T2 and T3. It was noted a decrease in SLA in the control group after the first time point. This finding may be explained by the observation that when the rat entered the arena for the first time, the behavior observed could be classified as "exploratory activity" in which the animal moves a lot exploring the new environment. At subsequent times, being already familiarized with the arena, the exploratory behavior ceases and the spontaneous locomotor activity reduces.

It can be seen in Figure 2 that levomepromazine, azaperone and midazolam caused a decrease in SLA when observed 8 min after drug administration (T1), characterizing a sedative effect for all the drugs studied. However, levomepromazine produced a more pronounced effect in comparison to the other drugs used at the subsequent time points. In addition, despite a lowering of the SLA found also in the control group animals, the effect of levomepromazine remained significantly lessened at times 2 and 3. These results agree with O'nell and Fountain (1999) who attributed antipsychotic, anxiolytic and sedative activities to levomepromazine.

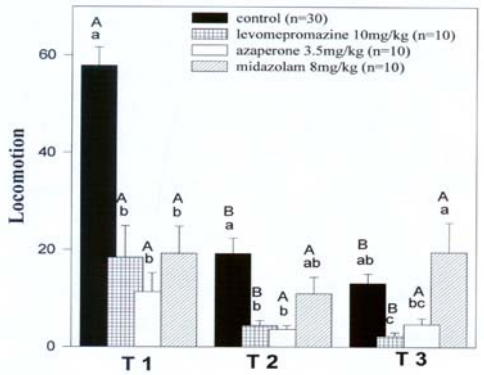

Figure 2. Measure of locomotor activity in rats at 3 time points after ip administration of saline (control), levomepromazine (10mg/kg), azaperone (3.5mg/kg), midazolam (8.0mg/kg). Vertical bars indicate SEM. Different upper-case letters indicate a difference ($P<0.05$) between times. Different lower-case letters indicate a difference ($P<0.05$) between groups.

Accordingly, the results of SLA test (Figure 2) showed that azaperone continued having a significant depressor effect in this test, quite evident even at T2, although being statistically not different at T3. This finding was possibly due to the reduction of SLA also in the control at T3. This drug when utilized alone can be employed in the treatment of various behavioral changes in many animal species (Olson and Renchko, 1988).

Midazolam significantly reduced the SLA, when compared to control values, only at T1, suggesting that in some form this result is related to exploratory behavior, which was evident in T1. However, the literature about therapeutic uses and toxicity of midazolam in humans (Nordt and Clark, 1997) describes that this drug has been used effectively in the rapid sedation of highly agitated patients, epilepsy and other behavior emergencies. Another feature of this drug includes a reduction in locomotion. Although, in this study, the reducton in SLA was not observed, we could notice changes in the exploratory behavior.

The antinociceptive effect of the tranquilizers utilized, determined by the increase in latency time of tail flick reflex (TFRL) in rats is illustrated in Figure 3. Levomepromazine, azaperone and midazolam did not produce a significant effect on nociception in rats.

Results of the levomepromazine dosage used in the present study do not agree with Hals and Dahl (1995) who showed that the drug is used in the treatment of behavioral disorders and also as an analgesic agent.

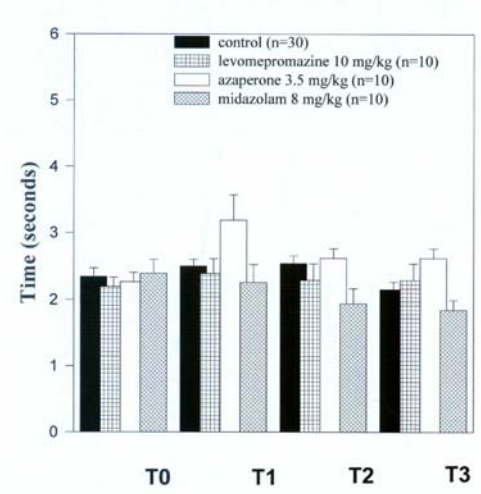

Figure 3. Measure of rat tail flic reflex latency at 3 time points after ip administration of saline (control), levomepromazine (10mg/kg), azaperone (3.50mg/kg) or midazolam (8.0mg/kg). T0 – time immediately before administration of drug, T1 – 10min, T2 – 20min and T3 – 30min after drug administration. Vertical bars indicate SEM. No significant differences between the control and treated groups were observed.

Olson and Renchko (1998) utilized 10mg/kg azaperone in a pain test in rats based on the withdrawal of the pelvic limb in response to a pain stimulus. In this study, azaperone demonstrated a reduction in the pelvic limb withdrawal reflex for more than 2h40min, proving a considerable antinociceptive effect. The absence of the antinociceptive effect can be related to the dose used in the present study (3,5mg/kg) wich was much lower than the dose used by Olson and Renchko (1998). These authors used 10mg/kg of azaperone in a test in rats based on the withdrawal of the pelvic limb in response to pain stimulus. In the present study, azaperone demonstrated a reduction in the pelvic limb withdrawal reflex for more than 2h40min, proving a considerable antinociceptive effect.

Results revealed in this study did not show any significative effect in TFRL when midazolam was administered. Therefore, they do not agree with those obtained by Kotinen and Dickenson (2000) who evaluated the pharmacological effect of midazolam in behavior studies of allodynia to determine possible changes in GABAergic activity in a model of neuropathic pain in rats.

Many experimental and clinical investigations on the modulation of GABAergic transmission in nociceptive processes and in the potential use of GABAergic agents used as analgesics have produced many conflicting results. Although several studies have demonstrated benzodiazepines as analgesics, other studies have emphasized that this drug does not possess any antinociceptive property (Clavier et al., 1992). Cartmell and Mitchell (1993) demonstrated that benzodiazepines attenuate hyperalgesia in ischemia-reperfusion of the rat tail artery.

This diversity in results is based on the variety of different experimental protocols, nociception models and modes of drug administration. Moreover, the sedative, anxiolytic and muscle-relaxing effects of GABAergic agents, and particularly those of benzodiazepines, can confuse the interpretation of behavioral and clinical studies (Kotinen and Dickenson, 2000).

Figure 4 shows the results related to the antinociceptive effects of levomepromazine, azaperone and midazolam in the writhing reflex in mice. In this test, all drugs examined were able to abolish the writhing reflex, establishing an appropriate antinociceptive effect with respect to visceral pain in mice, evoked by the injection of 0.6% acetic acid.

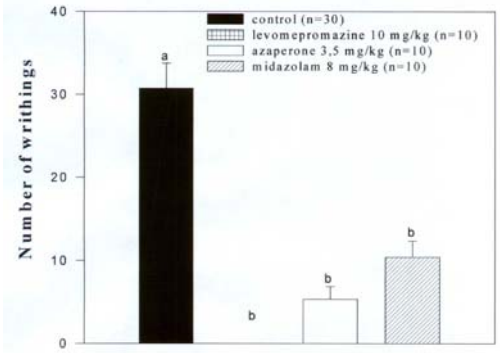

Figure 4. Antinociceptive effect of levomepromazine (10.0mg/kg), azaperone (3.50mg/kg) and midazolam (8.0mg/kg), evaluated by the writhing test in mice. The results show the number of writhings observed in 20min after ip administration of 0.6% acetic acid (V/V). Lower-case letters indicate a diference ($P<0.05$) between groups.

The effect of midazolan found in the present study, agrees with those related by Sierralta and Miranda (1993) who observed an additive effect of midazolan and adenosine-related compounds to produce a dose-dependent reduction in the number of writhings in mice. Regarding the results for the writhing test using levomepromazine and azaperone, it was not found any study that could compare the results obtained in this research. Nevertheless, these drugs provided a considerable analgesy related to visceral pain.

CONCLUSIONS

It can be concluded that levomepromazine, azaperone and midazolam, at the doses studied, were capable of inhibiting exploratory behavior in rats. Azaperone caused a more rapid effect on SLA in comparison with levomepromazine and midazolam. Regarding the antinociceptive effects, the drugs utilized were capable of impeding writhing in mice but were not able to induce any available effect on the tail flick reflex. The results present here indicate that depending on the method utilized to assess antinociceptive effects the results can vary. This observation should serve as an alert to researchers and other professionals who work with pain, as the fact than an animal does not react to pain does not always mean that it is not feeling any. What may happen is that the method used to assess pain may not be the most adequate one.

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