Pharmacology Biochemistry & Behavior, Vol. 13, pp. 443–447. Printed in the U.S.A.

Strain Dependent Effects of Ketamine on Locomotor Activity and Antinociception in Mice

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Received 6 March 1980

FILIBECK, U. AND C. CASTELLANO. *Strain dependent effects of ketamine on locomotor activity and antinociception in mice.* PHARMAC. BIOCHEM. BEHAV. 13(3) 443–447, 1980.—The effects of ketamine (12.5, 25 and 50 mg/kg) on locomotor activity and response to nociceptive stimuli were investigated in the inbred strains of mice: BALB/c (BALB), C57BL/6 (C57) and DBA/2 (DBA). In the BALB and in the C57 mice ketamine exerted activity stimulating effects, which were already present at doses lower than those inducing antinociception. Locomotor depressant effects were evident in the DBA mice following the administration of doses higher than those necessary to induce analgesia. It is suggested that: (1) ketamine affects locomotor activity and response to painful stimuli through different mechanisms, (2) the brain regional and biochemical differences reported for the strains considered may account for their different responses to ketamine administration.

Ketamine Activity Antinociception Inbred mice

KETAMINE (2-orthochlorophenyl-2-methylaminocyclohexanone HCl) is a dissociative anaesthetic, whose administration is followed, in man, by analgesia, anaesthesia, with restlessness and emergence phenomena during recovery [7]. Ketamine induced antinociception has also been described in laboratory animals, together with stimulation of locomotor activity [5,16].

In recent years the inbred strains of mice have proved to be a useful tool in psychopharmacological investigations. A number of studies have shown, for example, that the genetic make-up can play an important role in modulating the effects of opiates and hallucinogens on behavior, and have given useful information concerning their mechanisms of action [2, 4, 18].

In the present experiment three inbred strains of mice were used: BALB/c (BALB), DBA/2 (DBA), and C57BL/6 (C57), which differ with respect to behavior and brain chemicals. In particular, the C57 strain is characterized by high spontaneous locomotor activity (and low levels of avoidance and maze learning), while the other two display high avoidance and maze learning but lower general activity [3, 17, 18].

Some studies have shown moreover that these strains also differ when their levels and turnover of brain neurotransmitters are considered: the C57 mice are, in fact, characterized by lower acetylcholinesterase and choline-acetyltransferase activity in the temporal lobe, than the other two strains [8, 10, 15]. As for the adrenergic system, it has been shown that the C57 strain is characterized by higher levels of noradrenaline in the pons and medulla, while lower levels of this mediator seem to be positively correlated with the lower activity of the other strains [12]. Finally higher levels of serotonin in pons, medulla, midbrain and forebrain are present in the BALB strain, compared with the other two.

In the present research these strains were selected, on the basis of the above cited behavioral and neurochemical differences, in order to assess possible strain-dependent effects of ketamine on locomotor activity and antinociception.

METHOD

Subjects

The subjects were naive male mice belonging to the strain BALB/c, C57BL/6 and DBA/2 (River Lab., Como, Italy) weighing 23–25 g at the beginning of the experiment. All mice were maintained upon their arrival in the laboratory (2 weeks before the experiments) in groups of 5 in clear plastic pens with food and water available ad lib. In all experiments the animals were tested once.

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Locomotor Activity

Locomotor activity was measured as previously described [19]. The mice were tested in Plexiglas toggle-floor boxes (24.5×9.0 cm). The number of crossings from one side to the other of the box was automatically recorded by means of a microswitch connected to the tilting floor of the box, and constituted the score of the mouse. Circuitry was arranged so that whenever the mouse crossed the cage, a cumulative counter was advanced. A light located 1.5 m above the top of the boxes was the source of illumination (0.25 Ft-c. at the cage floor level). Since preliminary experiments had shown that the effects of ketamine in the strains tested are short lasting each group of mice was tested for a single session of 30 min.

Antinociception

The degree of antinociception was determined with the hot plate method, as previously described [19]. The endpoint used was the licking of forepaws or hindpaws. A mouse was removed as soon as it reacted or if it failed to react after 30 sec.

Experimental Schedule

Experiments were carried out according to a previously described experimental schedule [18].

Dose response curves for analgesia and locomotor activity were obtained by injecting the mice IP with ketamine HCl (Ketalar, Parke-Davis) diluted with 0.9% NaCl and injected at the volume of 4 ml/kg.

The animals were tested for activity 5 min after the injection. The locomotor activity was rated in a group of ten control mice, injected with saline (0.9% NaCl), and in three other different groups of ten mice per strain; each group being injected with a different dose of ketamine (12.5, 25 and 50 mg/kg).

In a separate series of experiments, the analgesic effect was studied. The reactivity to the hot plate was first measured in all subjects belonging to the three strains. Following the determination of reactivity in absence of drug, the mice were divided in groups of ten, and each group was used to assess the antinociceptive response to a different dose of ketamine (12.5, 25 and 50 mg/kg). The interval between the first and the second testing was 1 hr since preliminary experiments showed that the optimal interval of 1 hr did not implicate any interference between the two measures. Thus each group was tested at time zero, in the absence of drug (baseline), injected with ketamine, and retested to assess the degree of antinociception 5 min later, since preliminary experiments showed that the effects of ketamine are evident in the strains tested after 2–3 min. The recovery from the antinociceptive effect was assessed by testing each animal 20, 40 and 60 min after the injection.

For each strain two additional groups of 10 mice each were finally used to test the effects of ketamine vehicle on activity and antinociception respectively (at the concentration corresponding to the highest dose of ketamine injected).

The results were statistically evaluated by ANOVA [9] and by trend analysis. Additional analyses were also carried out when necessary in order to obtain individual between-treatment comparisons [14]

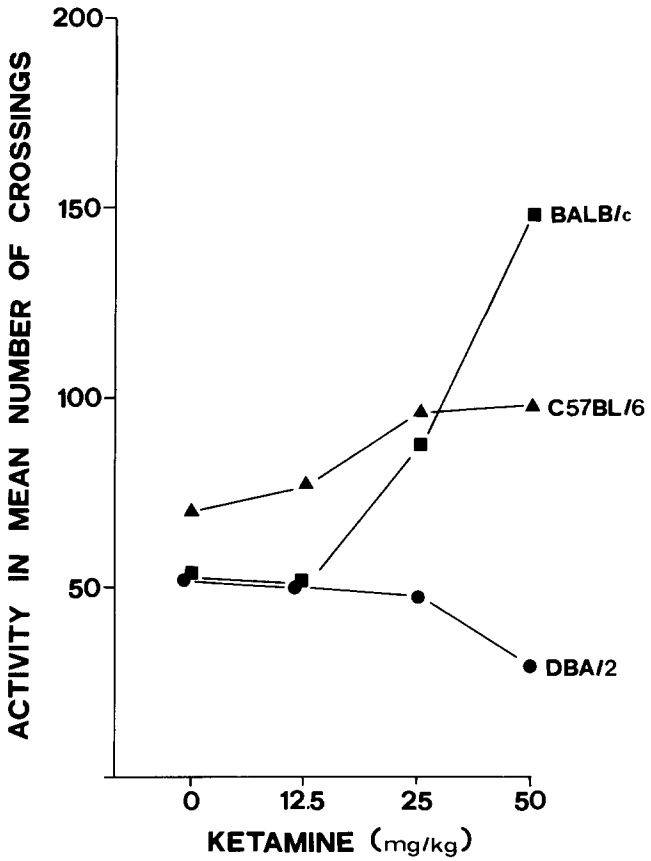

FIG. 1. Effects of different doses of ketamine on the locomotor activity of BALB/c, C57BL/6 and DBA/2 mice. Each group consisted of 10 mice tested 5 min following the injection of ketamine.

RESULTS

Locomotor Activity (Fig. 1)

The ANOVA, carried out for all experimental groups, showed significant differences between groups, $F(11,108)=29.0$, $p<0.001$.

In the additional analyses of individual between group comparisons the F values were tested for their significance on the basis of 1,108 df.

In the 0.9% NaCl injected mice significant differences were evident between the performances of the C57 and those of both the BALB ($F=3.97$, $p<0.05$) and the DBA mice ($F=4.03$, $p<0.05$). No difference was evident between the activity levels of the DBA and the BALB mice.

Ketamine administration was followed by locomotor stimulation in the BALB and the C57 mice, while locomotor depressant effects were evident in the DBA mice.

In particular, the administration of 12.5 mg/kg of ketamine to BALB mice did not significantly modify locomotor activity levels, compared to controls. Locomotor activity was significantly enhanced following the administration of both 25 mg/kg ($F=18.4$, $p<0.001$) and 50 mg/kg ($F=136.3$, $p<0.001$) of ketamine.

The locomotor activity of C57 mice was not significantly different from those of the 0.9% NaCl injected group following the administration of 12.5 mg/kg of ketamine. Locomotor activity was significantly enhanced following both 25 mg/kg

($F=9.54$, $p<0.01$) and 50 mg/kg ($F=10.6$, $p<0.01$) administrations.

No significant differences were evident between the activity levels of the DBA mice injected with 12.5 or 25 mg/kg of ketamine, and those of the 0.9% NaCl injected group. The administration of 50 mg/kg of ketamine was followed, in this strain, by a significant locomotor activity decrement ($F=10.3$, $p<0.01$).

No significant difference was evident in all strains between the activity levels of the vehicle injected mice and those of the saline injected controls.

Antinociception

The 0.9% NaCl injected mice of all three strains (when placed on the hot plate) licked their forepaws or hindpaws within a mean latency of 10–11 sec. Trend analysis showed in particular no significant differences between strains, $F(2,27)=0.27$, $p>0.05$, between times, $F(4,108)=1.87$, $p>0.05$ and no significant strains×times interaction, $F(8,108)=1.3$, $p>0.05$.

Ketamine administration produced antinociceptive effects in all strains. In this case trend analysis was carried out to determine which strain responses to the administrations of ketamine varied within each dose (12.5, 25 and 50 mg/kg) and at different times (5, 20, 40 and 60 min respectively) following the administration of the drug. Following the administration of 12.5 mg/kg of the drug, trend analysis showed, in particular, no significant differences between strains, $F(2,27)=0.27$, $p>0.05$, between times, $F(4,108)=1.87$, $p>0.05$ and no significant strains×times interaction, $F(8,108)=1.3$, $p>0.05$.

Following the administration of 25 mg/kg of ketamine trend analysis showed significant differences between strains, $F(2,27)=58.3$, $p<0.001$, between times, $F(4,108=49.4$, $p<0.001$, and also significant strains×times interaction, $F(8,108)=23$, $p<0.001$. In particular the reaction times of the C57 and the BALB mice did not significantly change, compared with the 0.9% NaCl injected controls. However, after ketamine the reaction times were significantly different from controls in the DBA strain, but only 5 and 20 min following the administration, $F(1,108)=23.4$ and 5.7, $p<0.001$ and $p<0.05$ respectively.

Following the administration of 50 mg/kg of ketamine, trend analysis showed significant differences between strains, $F(2,27)=62.1$, $p<0.001$, between times, $F(4,108)=103.0$, $p<0.001$, and also a significant strains×times interaction, $F=17.1$ $p<0.001$.

In the BALB strain of mice the reaction times were, in particular, significantly enhanced, as compared with the controls, only 5 min following ketamine administration, $F(1,108)=4.5$, $p<0.05$. In the DBA strain, a significant increase in reaction time was evident 5, 20 and 40 (not 60) min following injection, $F(1,108)=22.1$ ($p<0.001$), 28.8 ($p<0.001$) and 4.0 ($p<0.05$) respectively. In contrast the reaction times of C57 mice were significantly enhanced, as compared with 0.9% NaCl injected mice, but only 5 min following injection, $F(1,108)=4.0$, $p<0.05$).

Moreover, the mean latency of the DBA strain 5 min following the injection of 50 mg/kg of ketamine was significantly different from that of both the C57 and BALB strains $F(1,27)=55.9$ and 43.7, respectively, $p<0.001$. No significant difference was instead evident between the latency of the BALB and C57 mice $F(1,27)=0.7$, $p>0.05$.

Following recovery from the analgesic effect of ketamine the DBA mice reached the predrug latency values later than BALB and C57 mice (Table 1).

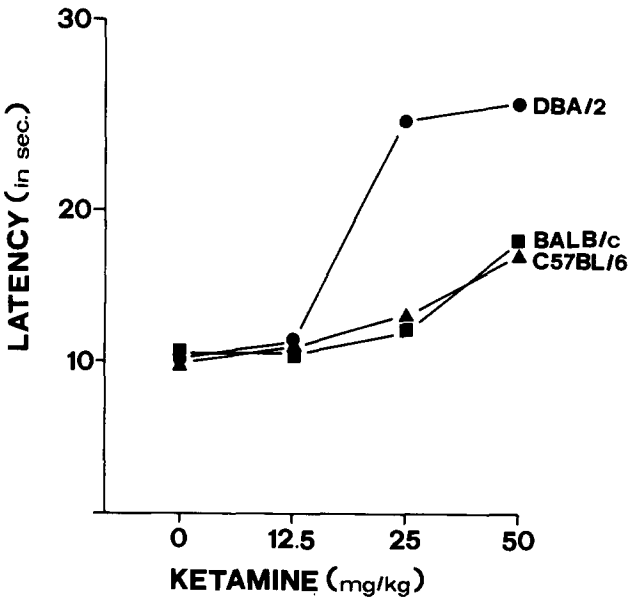

FIG. 2. Ketamine induced antinociception in DBA/2, BALB/c and C57BL/6 mice. The animals were tested in absence of drug (0), and 5 min following the injection with the different doses of the drug.

No significant difference was evident between the reaction times of the mice injected with ketamine vehicle and those of the baseline.

DISCUSSION

As far as the locomotor activity measures are concerned, the results of the present research show, in agreement with previous results [17], that the C57 mice are characterized by higher spontaneous locomotor activity compared with the BALB and the DBA mice, and that no significant differences were present between the basal activity levels of the latter two strains. Following ketamine administration dose related activity increments in the BALB and in the C57 strain were observed and the former strain was more sensitive to the locomotor stimulating effect of the drug. The locomotor activity levels of the DBA mice were, on the contrary, depressed following ketamine administration. The depressant effect became evident following the highest dose tested.

It must be stressed that visual observations of the behavior of the three strains of mice carried out in preliminary experiments did not reveal any stereotyped behavior following the administration of the drug.

With regard to the nociceptive properties of the hot plate similar response latencies characterized the undrugged mice belonging to the three strains, in agreement with previous results [19]. When the antinociceptive effects of ketamine were considered, strain differences in reactivity were again evident. In this case DBA mice were the most sensitive to the antinociceptive effect of the drug, while similar response latencies characterized the other two strains. It must also be noted that the time course of recovery from antinociception following ketamine administration was also strain dependent. In fact, the DBA mice reached the predrug value later than the other two strains.

TABLE 1

MODIFICATION OF REACTION TIMES AT DIFFERENT TIME INTERVALS FOLLOWING KETAMINE ADMINISTRATION IN THE HOT PLATE TEST

Strains	Times after injection (min)	Ketamine (mg/kg) 12.5	25	50
DBA	5	11.6 ± 0.3	26.6 ± 1.2	27.5 ± 1.0
	20	10.8 ± 0.4	18.5 ± 1.7	27.0 ± 1.1
	40	10.2 ± 0.5	11.1 ± 0.7	18.1 ± 1.7
	60	10.3 ± 0.3	10.8 ± 0.3	11.1 ± 0.3
BALB	5	10.5 ± 0.4	12.1 ± 0.8	18.3 ± 0.8
	20	10.6 ± 0.3	10.0 ± 0.5	14.4 ± 0.6
	40	10.2 ± 0.3	9.8 ± 0.5	11.9 ± 0.6
	60	10.5 ± 0.3	10.5 ± 0.3	11.0 ± 0.4
C57	5	11.1 ± 0.4	13.1 ± 0.8	17.1 ± 1.2
	20	10.1 ± 0.3	10.4 ± 0.3	13.8 ± 0.4
	40	10.6 ± 0.4	9.2 ± 0.3	11.6 ± 0.4
	60	10.5 ± 0.3	11.0 ± 0.4	10.4 ± 0.3

It is interesting to point out that the locomotor stimulating effects of ketamine were evident, in the BALB and the C57 mice, following the administration of doses which did not affect antinociception, while antinociception was evident in the DBA strain following doses lower than those necessary to depress locomotor activity. The lack of correlation between antinociceptive and activity effects of ketamine is also demonstrated by the fact that the BALB mice were more sensitive than the C57 mice to the locomotor stimulating effect of ketamine, while the two strains showed similar response latencies in the hot plate test.

The results of the present research clearly underline the role played by genetic factors in modulating the effects of ketamine on locomotor activity and antinociception in mice. They also suggest that these effects may be mediated through different mechanisms.

A number of researchers have investigated the effects of ketamine on levels and turnovers of brain mediators in the rat. It has been demonstrated [9] that this drug affects brain monoamine levels. Vargiu *et al.* [20] have shown that ketamine also increased serotonin turnover, and that inhibitors of its synthesis or serotonin receptor blockers potentiate the anaesthetic and antinociceptive effects of the drug. It has also been demonstrated that anticholinesterase agents can alter the duration of anaesthesia (measured by the loss of the righting reflex) without affecting the antinociceptive action of ketamine [13]. Since clear differences in the levels and turnovers of neurotransmitters have been demonstrated in the strains tested, the present findings suggest that these differences might be responsible for the strain dependent responses observed following ketamine administration.

In conclusion, the differential actions of ketamine on the behavioral measures studied support the existence of different receptors or systems responsible for the antinociceptive and the locomotor effects of this drug. It must be pointed out that the implication of different systems or receptors in the antinociceptive and the excitatory responses of mice and rats is evident for a number of analgesic agents ranging from opiates [19] to prostaglandins and D-amino acids [1,6]. The present paper extends this conclusion to ketamine as well.

ACKNOWLEDGEMENTS

We thank prof. Paolo Renzi for his helpful suggestions during the statistical evaluation of the results, and Mr. Giuseppe Olimpieri for his skillful technical assistance. This work was supported by a grant from the Italian Ministry of Health.

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