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RAPID COMMUNICATION

Role of Dopamine Receptors in the Occurrence of the Behavioral Motor Disturbances in Rats Exposed to High Pressure

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ABRAINI, J. H., C. TOMEI AND J. C. ROSTAIN. *Role of dopamine receptors in the occurrence of the behavioral motor disturbances in rats exposed to high pressure.* PHARMACOL BIOCHEM BEHAV **39**(3) 773–779, 1991.—When human divers and experimental animals are exposed to an increasing environmental pressure, they develop the high pressure neurological syndrome (HPNS) characterized by electroencephalographic changes and sleep and behavioral disturbances. In rats, behavioral disturbances essentially include hyperlocomotor activity (HLA), tremor and myoclonia. Moreover, HLA has recently been demonstrated to be linked to a pressure-induced striatal increase of dopamine (DA). In these experiments, it was proposed to investigate in rats, at the behavioral level, the role of DA receptors in the occurrence of the pressure-induced DA disturbances. DA receptor agonists were found to induce no significant changes in the development of HLA, tremor, and myoclonia. Alternatively, HLA was found to be dramatically antagonized by the use of DA receptor antagonists (SCH 23390, sulpiride, and haloperidol), while tremor and myoclonia only decreased in SCH 23390 experiments.

Neuroleptics Locomotor activity High pressure neurological syndrome Dopamine

HIGH pressure is known as a basic etiological factor underlying central nervous system (CNS) changes referred to as high pressure neurological syndrome (HPNS). This syndrome is observed when human divers or experimental animals are exposed to high pressure >20 bar.

The principal symptoms include EEG changes, sleep disturbances, muscle tremor, and myoclonia. Animals also developed hyperlocomotor activity (HLA), and for higher pressures, convulsions and epileptic seizures. These symptoms have been described by several authors (5, 6, 8, 19), and reviewed elsewhere (9). In man, other effects include problems of motor coordination and loss of attention (12). In divers exposed to hyperbaric experiments, recent data have reported psychotic disorders such as schizophrenic symptoms of paranoid form with delirium and ambulatory activities (15, 18, 20, 22), which are generally considered as the result of a dopamine (DA) hyperactivity [for review see LeMoal (13)].

Several in vitro and in vivo studies have demonstrated that high pressure induced an increase in extracellular striatal DA level (1, 2, 7, 14, 16), and moreover, the development of HLA has been found to be linked to the pressure-induced striatal DA increase. Furthermore, in a recent study, we experimentally suggested that the mechanisms involved in the pressure-induced striatal DA increase would be similar to some of those involved in psychosis/schizophrenia. Consequently, we speculated that the use of DA receptor antagonists (i.e., of neuroleptics) could be of strong interest in the treatment of HLA (Abraini et al., in preparation.)

In this field of investigation, we report here pharmacological experiments on the effects of the use of DA receptor agonists (SKF 38393, a D1 receptor agonist, LY 171555, a D2 receptor agonist, and apomorphine, a D1/D2 receptor agonist) and antagonists (SCH 23390, a D1 receptor antagonist, sulpiride, a D2 receptor antagonist, and haloperidol, a D1/D2 receptor antagonist) on the development of the behavioral motor disturbances, including HLA, tremor, and myoclonia, in free-moving rats exposed to high pressure. Drugs were administered intracerebroventricularly (ICV) because of technical data such as the administration of drugs in free-moving rats through a closed chamber.

METHOD

Animals

Male Sprague-Dawley rats (n = 30) weighing 300–350 g at time of surgery were used. Rats were housed at 21 ± 0.5°C in individual altuglass home cages under a 12-12 h light-dark cycle (lights on from 7 a.m. to 7 p.m.), with free access to food and water.

Drug Treatment

Pharmacological compounds were administered ICV through a stainless steel cannula which was stereotaxically and chronically implanted, under general anesthesia (pentobarbital sodium 30 mg/kg IP and ketamine 100 mg/kg IM), in the right lateral ventricule of the animals (A: 5.91; L: 1.4; H: 2) according to the atlas of König and Klippel (11), and held in place with dental cement. After surgery, the animals were allowed to recover one week before the pharmacological investigations.

Pharmacological compounds were delivered in 10 µl of phosphate buffered saline solution (PBS) at 10^{-8} M, i.e., 5 µg/kg SKF 38393 (n=4); 6 µg/kg LY 171555 (n=4); 6 µg/kg APO (n=4); 6 µg/kg SCH 23390 (n=4); 7 µg/kg sulpiride (n=4); and 7 µg/kg haloperidol (n=4). During previous ICV experiments at atmospheric pressure, these doses had been found to alter extracellular DA release over a period of time of more than 24 h (3). DA receptor agonists and antagonists were purchased from Research Biochemicals Incorporated (Natick, MA). Control experiments were performed in animals injected with 10 µl PBS (n=6).

Exposure to Pressure

The free-moving animals were placed in separate altuglass cylinders in a 50-liter pressure chamber (maximum pressure 200 bar) in which the 12-12 h light-dark regime was maintained. Rats were compressed to 80 bar of relative pressure (equivalent of 800 m of sea water) with helium, at a rate of 1 bar/min, 1 h 30 min after being injected with PBS or drugs. Oxygen was maintained at a constant partial pressure of 0.4 bar, which is the partial pressure generally used for human divers. The CO_2 was less than 0.0003%. Humidity was controlled, and temperature was progressively increased from 25 to 33°C to prevent hypothermia, because of the important specific heat of helium as compared to air, and to maintain the comfort of the animals. The stay at the maximal depth lasted 4 h, and the decompression 24 h. Animals were decompressed at a rate of 0.06 bar/min from 80 to 12 bar and 0.04 bar/min from 12 bar to atmospheric pressure. During decompression, partial pressure of oxygen was 0.5 bar. All the animals survived the hyperbaric experiments.

Behavioral Analysis

Behavioral analysis was performed as described previously by Tomei et al. (23). Principles of analysis can be summarized as follows: behavioral symptoms of HPNS were obtained from piezoelectrical sensors which were fixed under the floor of each altuglass cylinder; signals were quantitatively analyzed on a PC-AT compatible computer, and decomposed on line in HLA, tremor and myoclonia. Tremor was calculated from the signals recorded on the 10–16 Hz band frequency (4,24); myoclonia was detected as signals of unusual high amplitude (with a threshold of detection adjustable for each rat); and HLA as the whole signal minus myoclonia and tremor. HLA, tremor, and myoclonia were expressed in arbitrary units (U).

Statistical Methods

Nonparametric statistics were used such as the Wilcoxon sign-rank paired *t*-test (W-test), the Mann-Whitney U-test (U-test) and median value ± the 25th–75th percentiles. W-test was used for control experiments to analyze the effect of pressure on the rat's behavior (before and after pressure exposure); U-test was used to compare data obtained in injected animals during drug experiments to the corresponding data (same pressure during compression and decompression, and same time during the 4-h stay at 80 bar) recorded in animals injected with PBS during control experiments (21).

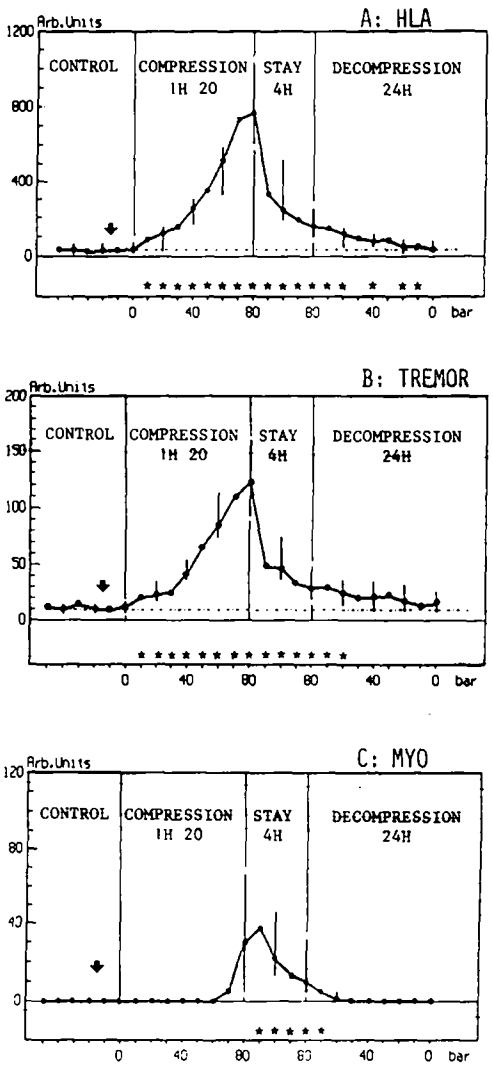

FIG. 1. Development of the pressure-induced behavioral motor disturbances during pressure experiments in free-moving rats exposed to 80 bar (n=6). (A) hyperlocomotor activity (HLA); (B) tremor; (C) myoclonia. Left: compression up to 80 bar, duration 1 h 20 min. Middle: stay at 80 bar, duration 4 h. Right: decompression from 80 bar to atmospheric pressure, duration 24 h. Y-axis: motor disturbances expressed in arbitrary units; median values ± 25th–75th percentiles. X-axis: pressure expressed in bar, 1 bar = 10^5 P; representation is not proportional to time. W-test: *$p<0.05$. Arrows indicate time of injection of PBS saline solution (1 h 30 min before pressure exposure).

RESULTS

Effects of High Pressure on Motor Behavior

Compression was found to lead to a sustained increase of HLA (maximal value: 770 U, W-test: $p<0.05$), tremor (maximal value: 122 U, W-test: $p<0.05$) and myoclonia (maximal value: 37.5 U, W-test: $p<0.05$). During the 4-h stay and de-

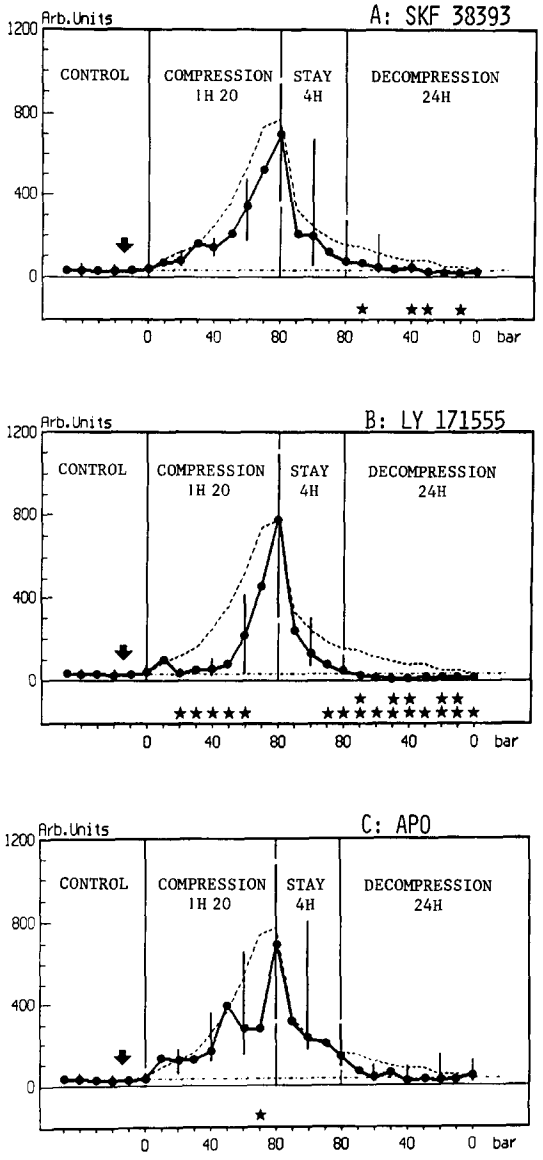

FIG. 2. Development of hyperlocomotor activity during pressure experiments in free-moving rats exposed to 80 bar and injected with DA receptor agonists. Left: compression up to 80 bar, duration 1 h 20 min. Middle: stay at 80 bar, duration 4 h. Right: decompression from 80 bar to atmospheric pressure, duration 24 h. Y-axis: hyperlocomotor activity expressed in arbitrary units; median values ± 25th–75th percentiles. X-axis: pressure expressed in bar, 1 bar = 10^5 P; representation is not proportional to time. (A) In rats injected with SKF 38393 (n = 4); (B) in rats injected with LY 171555 (n = 4); (C) in rats injected with apomorphine (n = 4). U-test: $^{**}p<0.01$, $^{*}p<0.05$, vs. PBS experiments (dotted line). Arrows indicate time of injection (1 h 30 min before pressure exposure).

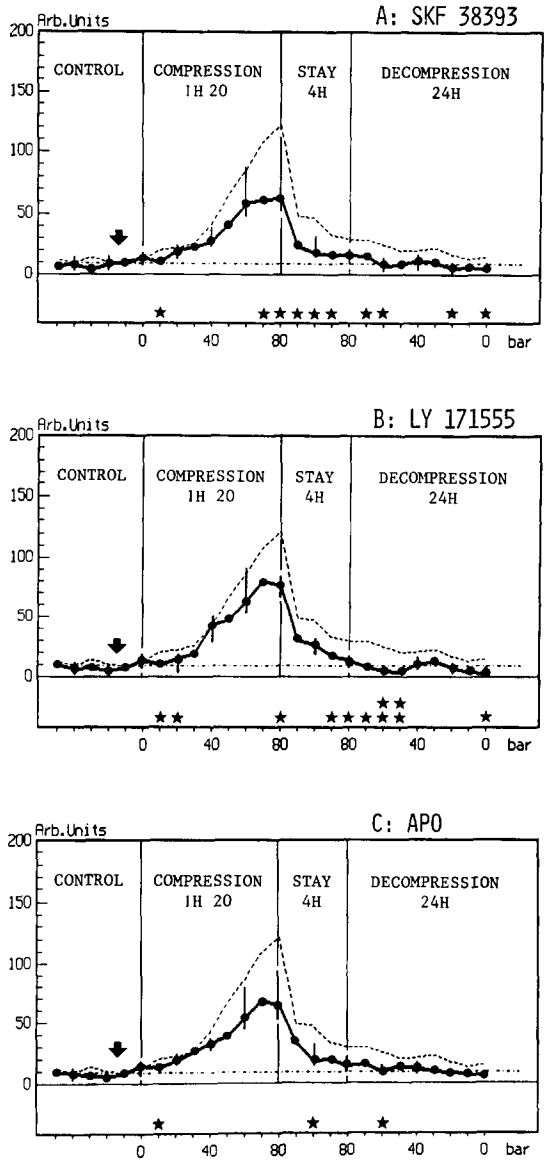

FIG. 3. Development of tremor during pressure experiments in free-moving rats exposed to 80 bar and injected with DA receptor agonists. Left: compression up to 80 bar, duration 1 h 20 min. Middle: stay at 80 bar, duration 4 h. Right: decompression from 80 bar to atmospheric pressure, duration 24 h. Y-axis: tremor expressed in arbitrary units; median values ± 25th–75th percentiles. X-axis: pressure expressed in bar, 1 bar = 10^5 P; representation is not proportional to time. (A) In rats injected with SKF 38393 (n = 4); (B) in rats injected with LY 171555 (n = 4); (C) in rats injected with apomorphine (n = 4). U-test: $^{**}p<0.01$, $^{*}p<0.05$, vs. PBS experiments (dotted line). Arrows indicate time of injection (1 h 30 min before pressure exposure).

compression phases, HLA, tremor, and myoclonia were found to decrease, and to progressively return to basal values (Fig. 1).

Effect of DA Receptor Agonists on the Behavioral Motor Disturbances

Administration of SKF 38393 or apomorphine was found to have no significant effect on the occurrence and the development of HLA (maximal value of HLA, SKF 38393: 696 U; apomorphine: 704 U), while the injection of LY 171555 strongly and significantly delayed the occurrence of HLA from 10 to 60 bar (U-test, $p<0.05$). Nevertheless, in spite of the administration of LY 171555, HLA was found to occur suddenly at 60–70 bar and to approximately reach at 80 bar the same value as the one recorded during PBS control experiments (maximal increase of HLA: 792 U, n.s.) (Fig. 2). Administration of SKF 38393, LY

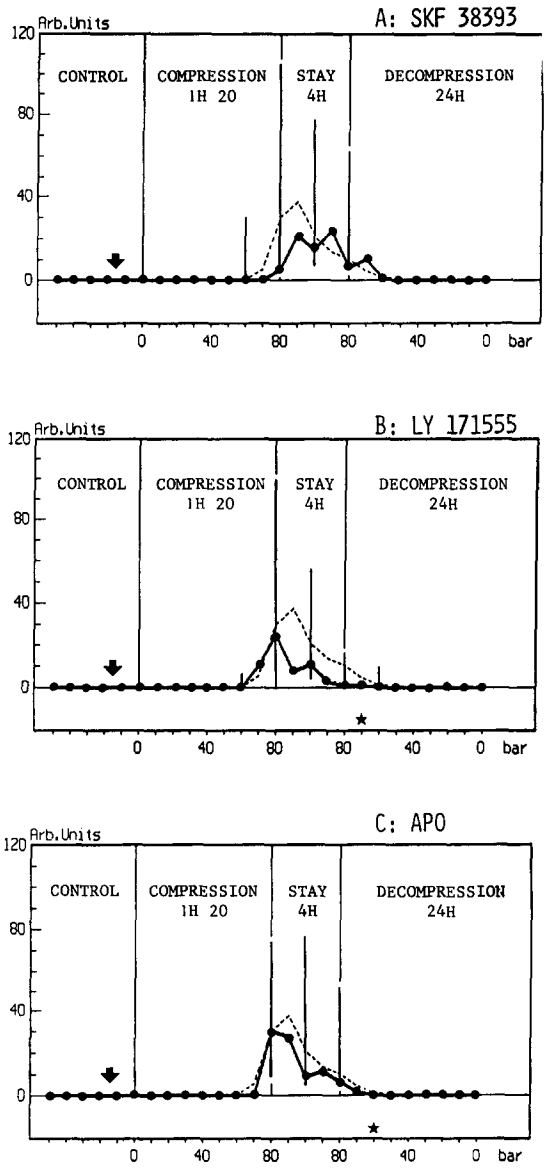

FIG. 4. Development of myoclonia during pressure experiments in free-moving rats exposed to 80 bar injected with DA receptor agonists. Left: compression up to 80 bar, duration 1 h 20 min. Middle: stay at 80 bar, duration 4 h. Right: decompression from 80 bar to atmospheric pressure, duration 24 h. Y-axis: tremor expressed in arbitrary units; median values ± 25th–75th percentiles. X-axis: pressure expressed in bar, 1 bar = 10^5 P; representation is not proportional to time. (A) In rats injected with SKF 38393 (n = 4); (B) in rats injected with LY 171555 (n = 4); (C) in rats injected with apomorphine (n = 4). U-test: *$p<0.05$ vs. PBS experiments (dotted line). Arrows indicate time of injection (1 h 30 min before pressure exposure).

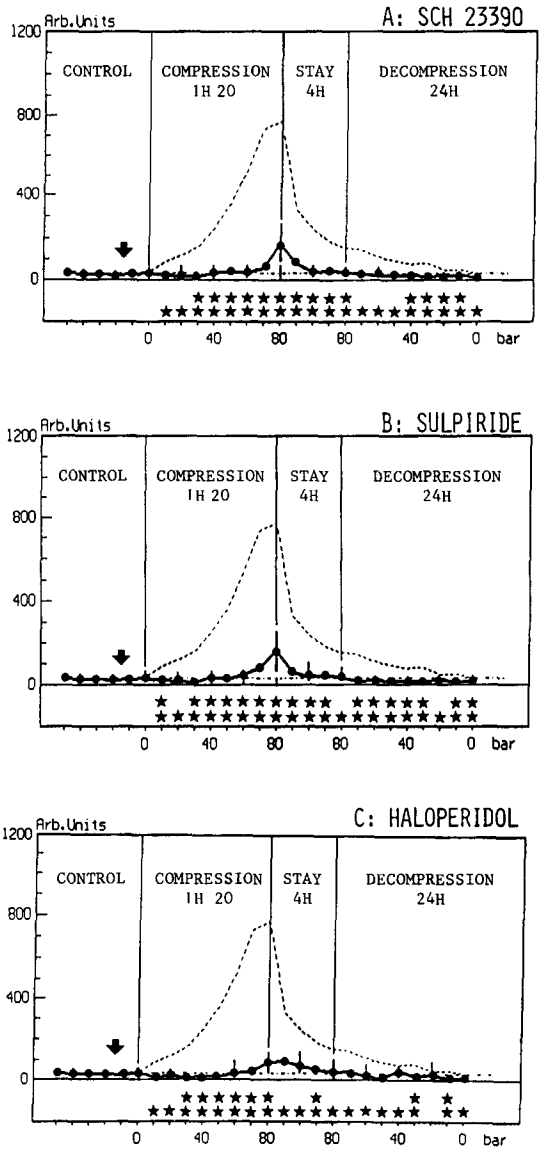

FIG. 5. Development of hyperlocomotor activity during pressure experiments in free-moving rats exposed to 80 bar and injected with DA receptor antagonists. Left: compression up to 80 bar, duration 1 h 20 min. Middle: stay at 80 bar, duration 4 h. Right: decompression from 80 bar to atmospheric pressure, duration 24 h. Y-axis: hyperlocomotor activity expressed in arbitrary units; median values ± 25th–75th percentiles. X-axis: pressure expressed in bar, 1 bar = 10^5 P; representation is not proportional to time. (A) In rats injected with SCH 23390 (n = 4); (B) in rats injected with sulpiride (n = 4); (C) in rats injected with haloperidol (n = 4). U-test: **$p<0.01$, *$p<0.05$, vs. PBS experiments (dotted line). Arrows indicate time of injection (1 h 30 min before pressure exposure).

171555, and apomorphine was found to have no significant effect on the occurrence and the development of tremor (Fig. 3) and myoclonia (Fig. 4). Furthermore, in some animals, the development of myoclonia was found to be very severe as compared to PBS control experiments.

In all cases, during the stay and decompression phases, the whole symptoms were found to progressively return to basal values.

Effect of DA Receptor Antagonists on the Behavioral Motor Disturbances

Administration of DA receptor antagonists (SCH 23390, sulpiride, and haloperidol) was found to dramatically and significantly decrease HLA as compared to PBS control experiments, but no major and significant differences were found between the effects of the different DA receptor antagonists that we used

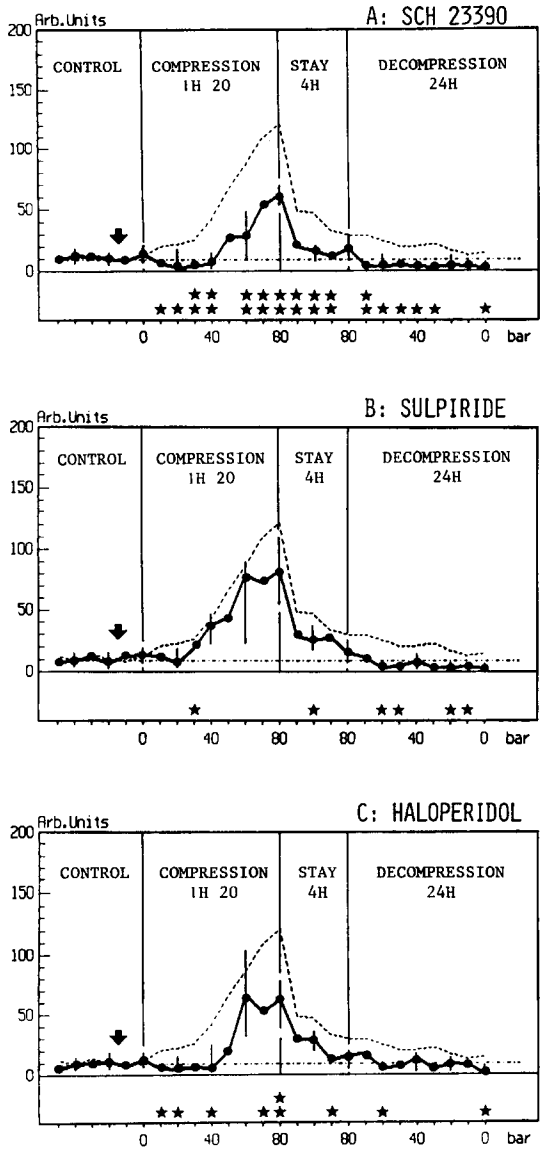

FIG. 6. Development of tremor during pressure experiments in free-moving rats exposed to 80 bar and injected with DA receptor antagonists. Left: compression up to 80 bar, duration 1 h 20 min. Middle: stay at 80 bar, duration 4 h. Right: decompression from 80 bar to atmospheric pressure, duration 24 h. Y-axis: tremor expressed in arbitrary units; median values ± 25th–75th percentiles. X-axis: pressure expressed in bar, 1 bar = 10^5 P; representation is not proportional to time. (A) In rats injected with SCH 23390 (n = 4); (B) in rats injected with sulpiride (n = 4); (C) in rats injected with haloperidol (n = 4). U-test: $^{**}p<0.01$, $^{*}p<0.05$, vs. PBS experiments (dotted line). Arrows indicate time of injection (1 h 30 min before pressure exposure).

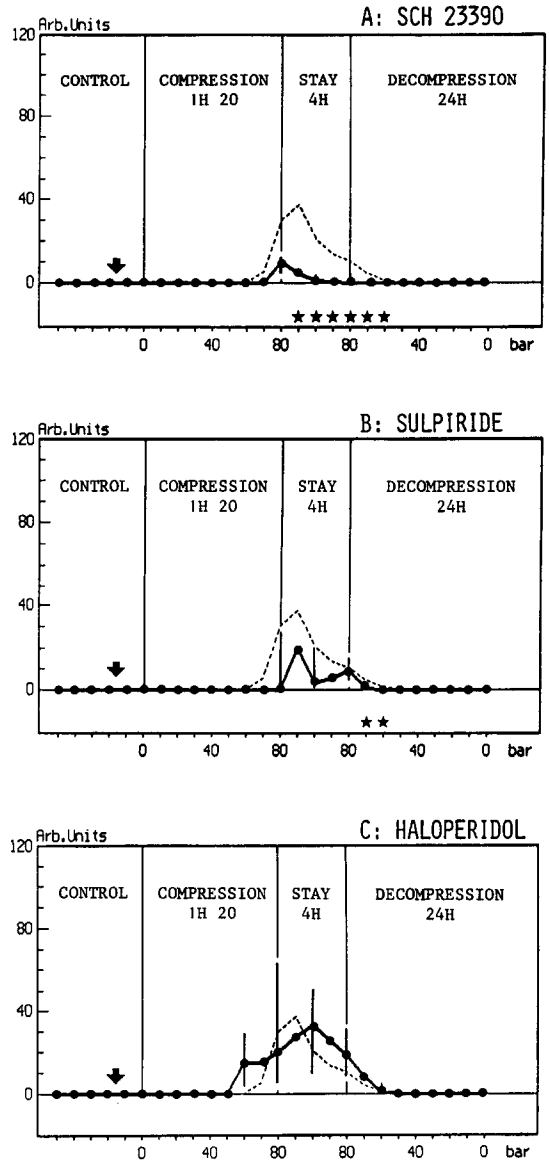

FIG. 7. Development of myoclonia during pressure experiments in free-moving rats exposed to 80 bar injected with DA receptor antagonists. Left: compression up to 80 bar, duration 1 h 20 min. Middle: stay at 80 bar, duration 4 h. Right: decompression from 80 bar to atmospheric pressure, duration 24 h. Y-axis: tremor expressed in arbitrary units; median values ± 25th–75th percentiles. X-axis: pressure expressed in bar, 1 bar = 10^5 P; representation is not proportional to time. (A) In rats injected with SCH 23390 (n = 4); (B) in rats injected with sulpiride (n = 4); (C) in rats injected with haloperidol (n = 4). U-test: $^{*}p<0.05$ vs. PBS experiments (dotted line). Arrows indicate time of injection (1 h 30 min before pressure exposure).

(maximal values of HLA, SCH 23390: 176 U, U-test $p<0.01$; sulpiride: 162 U, U-test $p<0.01$; haloperidol: 90 U, U-test $p<0.01$) (Fig. 5).

Administration of sulpiride and haloperidol was found to have no significant effect on the development of tremor (Fig. 6B and C) and myoclonia (Fig. 7B and C) during the compression phase of the pressure exposure. Alternatively, SCH 23390 was found to significantly decrease the development of tremor (maximal value: 64 U, U-test: $p<0.01$) (Fig. 6A) and myoclonia (maximal value: 4.5 U, U-test: $p<0.05$) (Fig. 7A) as compared to PBS control experiments.

As described above, the whole symptoms were found to progressively return to basal values during the stay and decompression phases of the pressure exposure.

DISCUSSION

The major finding of the present experiments was to demonstrate that the use of DA receptor antagonists strongly enables the counteraction of the development of HLA in rats exposed to high pressure, as we suggested previously in a recent neurochemical study performed to assess the mechanisms of the pressure-induced striatal DA increase (data not shown).

During PBS control experiments, compression was found to lead to a sustained increase of HLA, tremor, and myoclonia. These symptoms were found to progressively decrease during the 4-h stay and decompression phases, as described in previous studies (23). Administration of SKF 38393 or apomorphine was found to have no significant effects on the occurrence and the development of HLA, tremor, and myoclonia, while the administration of LY 171555 significantly delayed, but did not decrease the development of HLA, without, however, any effect on tremor and myoclonia. On the other hand, in all cases, the use of DA receptor antagonists (SCH 23390, sulpiride, and haloperidol) was found to significantly and dramatically decrease HLA, while essentially only SCH 23390 significantly altered the development of tremor and myoclonia.

The fact that DA receptor agonists did not increase HLA as compared to PBS control experiments suggests that the activation of DA receptors during the PBS pressure-induced locomotor activity could be almost at a maximal state of activation. Elsewhere, since it has been demonstrated that the activation of D2 postsynaptic receptors required the activation of D1 receptors (25), the delay observed in the occurrence of HLA after the administration of LY 171555, a specific D2 receptor agonist, would be the consequence of an inhibition of DA synthesis via the D2 presynaptic receptors. Nevertheless, in spite of the delay observed in the occurrence of HLA, the administration of LY 171555 failed to counteract the development of HLA. This result is in total agreement with a previous study in which we demonstrated similar phenomena by directly inhibiting DA synthesis using a-methyl-p-tyrosine (data not shown). These data reinforce previous behavioral and neurochemical works in which we suggested that DA postsynaptic receptors would play a preponderant role in the development of the pressure-induced striatal DA increase and its related symptom HLA (data not shown).

Alternatively, all the DA receptor antagonists that we used in the present experiments were found to dramatically and significantly decrease HLA as compared to PBS control experiments, and no significant difference was found between the effects of SCH 23390, sulpiride, and haloperidol on the development of HLA. The potent effect of these compounds confirms, at the behavioral level, the preponderant role of the DAergic pathways in the development of HLA, and furthermore, the crucial involvement of DA receptors in both the occurrence of this symptom and the pressure-induced DA disturbances. On the other hand, administration of SCH 23390 and sulpiride was found respectively to significantly and slightly decrease myoclonia, while the use of haloperidol slightly enhanced this behavioral motor disturbance as compared to control experiments. These results could be in relationship with the neuroleptic extrapyramidal side effects, since SCH 23390 and sulpiride are well known to be atypical neuroleptics, and haloperidol, at the opposite, a typical one. Elsewhere, hyperbaric tremor, which has been demonstrated to consist essentially of intention tremor, i.e., associated with voluntary movement activity [for review see (9)], was found to be decreased only in SCH 23390 experiments, despite locomotor activity also decreasing in sulpiride and haloperidol experiments. This suggests that the present data of tremor could be also linked to the extrapyramidal side effects of neuroleptics. However, the neuronal mechanisms and the neurochemical basis of tremor and myoclonia remain unknown.

In previous behavioral and neurochemical studies in which we demonstrated: (a) HLA included hoarding behavior (1); (b) the pressure-induced DA increase was higher in the nucleus accumbens than in the caudate-putamen (2); we suggested that HLA could be in part the expression of emotional disorders since the DA mesolimbic pathways have been demonstrated to be involved in hoarding behavior (10) and locomotion (17). The present data which demonstrate the potent effect of neuroleptics to counteract the development of HLA could reinforce this hypothesis. Nevertheless, at the present time, it is not possible to clearly determine whether HLA essentially consists in emotional disorders or not and thus further neurochemical and behavioral experiments, using for example focal injections of neuroleptics, are needed to assess the respective roles of the DA nigrostriatal and mesolimbic pathways in the occurrence of HLA.

In conclusion, this is the first study, to our knowledge, to report such a dramatic neuroleptic-induced decrease in HLA in rats exposed to high pressure, and to clearly demonstrate, at the behavioral level, the crucial role of DA receptors in both the occurrence of HLA and the pressure-induced DA disturbances. Nevertheless, further experiments are needed to thoroughly determine the neurochemical mechanisms of the neuronal and behavioral DAergic disturbances which occur under pressure.

ACKNOWLEDGEMENTS

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