Interference Between Central Dopaminergic Stimulation, and Adrenal Secretion in Normoxic or Hypobaric Hypoxic Rats

C. SALIGAUT, M.D., N. MOORE, M.D., P. CHRETIEN, M.D., M. DAOUST, M.D., O. RICHARD, M.D., AND F. BOISMARE, PH.D., M.D.

SUMMARY Previous data have established that postsynaptic stimulation of central dopaminergic receptors was mainly involved in the protective action of apomorphine against the compartmental consequences of hypobaric hypoxia in rats: disturbances in a conditioned avoidance response. We confirm this notion by showing that domperidone (a peripheral dopaminergic blocking agent) does not antagonize the protective effect of apomorphine. Furthermore, we establish that the action of apomorphine is at least partially mediated by adrenal glands since it is no longer seen in adrenalectomized rats. In normal rats, apomorphine enhances the corticosterone increase which is observed during hypobaric hypoxia and decreases the hypothalamic-pituitary-adrenal axis.

It is therefore concluded that the anti-hypoxic activity of apomorphine is probably mediated by a centrally mediated dopaminergic modification of the adrenal response to hypobaric hypoxia.

PREVIOUS RESULTS have established that iterative acute hypobaric hypoxia reduces avoidance response in rats.1-3

The dopaminergic agonists — piribedil, amantadine, bromocriptine and apomorphine4,5-8 increase the rats learning in hypoxic conditions. This protection is suppressed by a centrally-acting dopaminergic antagonist (Pimozide) at doses that block the post-synaptic receptors.2 In order to confirm that the protection afforded by apomorphine is mainly centrally mediated we have studied the interaction of apomorphine with a dopaminergic antagonist which does not cross the blood brain barrier: domperidone.6

It is well known that hypobaric hypoxia induces a stress, resulting in an increased cerebral5,7 and cardiac6 amine activity and in a modification of the adrenal metabolism.9,10 We then compared the effects of apomorphine in normal and adrenalectomized rats to see
whether there is an interaction of the hypoxia-induced reaction of adrenal glands with apomorphine’s effects.

Material and Methods

Study of the Conditioned Avoidance Response (CAR)

All experiments were performed on female Long Evans rats weighing 220 ± 20 g (groups of 10 animals). Hypoxia is obtained with a hypobaric chamber (Ateliers et Chantiers de Bretagne) in which a pressure of 300 torr, corresponding to an altitude of 7180 m, is obtained in 3 min and maintained during conditioning.

The sound avoidance conditioned reflex is induced in naive rats, at either 760 or 300 torr, with electronically controlled conditioning material, set inside the hypobaric chamber. Each animal is put inside the conditioning cage 5 min before the start of the experiment, so that it may explore all the environment possibilities. In the cage is a mast on which the animal can climb, an electrifiable floor with which the 20-s electric shock is given, and an electronic program with a regular cycle excluding any intervention by the experimentators. The animal itself stops the cycle by climbing on the mast; when it comes down from the mast, the cycle resets itself and starts all over again. The animals can stop the cycle by climbing on the mast at two different moments — during the electric shock (the rat has then acquired avoidance), or between the sound and the shock (5 s interval), the animal having then acquired avoid-

Table 1. Avoidance (CAR) + Escape (ESC) Performances During 5 Days of a Conditioned Avoidance Test for Normoxic or Hypobaric Hypoxic Rats (n = 10 per group). For Treatments See Materials and Methods.

<table>
<thead>
<tr>
<th></th>
<th>760 Torr</th>
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<tbody>
<tr>
<td>CAR + ESC</td>
<td></td>
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<tr>
<td>Control</td>
<td>67 ± 10</td>
<td>51 ± 9</td>
<td>98 ± 2</td>
<td>90 ± 6</td>
<td>99 ± 1</td>
<td>99 ± 1</td>
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<tr>
<td>Apomorphine</td>
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<tr>
<td>1 mg x kg⁻¹</td>
<td>21 ± 6‡</td>
<td>28 ± 10*§</td>
<td>46 ± 12†</td>
<td>52 ± 11*§</td>
<td>67 ± 9*</td>
<td>58 ± 10†§</td>
</tr>
<tr>
<td>Domperidone</td>
<td>66 ± 17</td>
<td>62 ± 9§</td>
<td>82 ± 18</td>
<td>99 ± 1§</td>
<td>84 ± 14</td>
<td>99 ± 1§</td>
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<tr>
<td>Apomorphine</td>
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<tr>
<td>1 mg x kg⁻¹</td>
<td>41 ± 11</td>
<td>58 ± 4</td>
<td>80 ± 11</td>
<td>84 ± 6</td>
<td>95 ± 3</td>
<td>94 ± 3</td>
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<tr>
<td>+ domperidone</td>
<td>NS</td>
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<td>Apomorphine</td>
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<tr>
<td>1 mg x kg⁻¹</td>
<td>24 ± 7‡</td>
<td>7 ± 4§</td>
<td>48 ± 14‡</td>
<td>8 ± 6‡</td>
<td>52 ± 12‡</td>
<td>12 ± 8§</td>
</tr>
<tr>
<td>+ adrenalectomy</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</tr>
</tbody>
</table>

Statistical analysis: 1) Student t test in reference with normoxic control rats: NS = not significant, *p < 0.05, †p < 0.01, ‡p < 0.001. 2) Potentialization test: $Simple conjunction of the effects of the treatments (drug + hypoxia, drug + drug + hypoxia) in reference with normoxic control rats. | Performances after association of the treatments are lower than the performances theoretically resulting of the simple conjunction of the treatments administered alone at statistical level: α < 0.23 for 3 treatments. ▲Performances after association are smaller than the performances resulting theoretically of the simple conjunction of the treatments administered alone: Statistical level: α < 0.19 for 2 treatments; α < 0.23 for 3 treatments.

Table 2. Avoidance (CAR) Performances During 5 Days of a Conditioned Test for Normoxic (760 Torr) or Hypobaric Hypoxic (300 Torr) Rats (n = 10 per group). For Treatments See Materials and Methods.

<table>
<thead>
<tr>
<th></th>
<th>760 Torr</th>
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</thead>
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<td>CAR</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>34 ± 8</td>
<td>6 ± 4†</td>
<td>60 ± 4</td>
<td>12 ± 5§</td>
<td>74 ± 6</td>
<td>27 ± 9§</td>
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<tr>
<td>Apomorphine</td>
<td>21 ± 6</td>
<td>4 ± 2†§</td>
<td>43 ± 6*</td>
<td>15 ± 5§</td>
<td>71 ± 6</td>
<td>37 ± 9†§</td>
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<tr>
<td>Domperidone</td>
<td>11 ± 4*</td>
<td>2 ± 2‡§</td>
<td>53 ± 11</td>
<td>16 ± 6§</td>
<td>62 ± 8</td>
<td>48 ± 13§</td>
</tr>
<tr>
<td>Apomorphine</td>
<td>19 ± 6</td>
<td>4 ± 3‡§</td>
<td>41 ± 5†</td>
<td>35 ± 8*</td>
<td>71 ± 6</td>
<td>52 ± 9§</td>
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<tr>
<td>+ domperidone</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Apomorphine</td>
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<tr>
<td>+ adrenalectomy</td>
<td>7 ± 4†</td>
<td>2 ± 1‡§</td>
<td>20 ± 6*</td>
<td>2 ± 1‡</td>
<td>23 ± 7‡</td>
<td>2 ± 1‡§</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>7 ± 3†</td>
<td>7 ± 4‡</td>
<td>39 ± 8*</td>
<td>17 ± 7§</td>
<td>59 ± 10</td>
<td>17 ± 7§</td>
</tr>
</tbody>
</table>

Statistical analysis: 1) Student t test in reference with normoxic control rats: NS = not significant, *p < 0.05, †p < 0.01, ‡p < 0.001. 2) Potentialization test: $Simple conjunction of the effects of the treatments (drug + hypoxia, drug + drug + hypoxia) in reference with normoxic control rats. | Performances after association of the treatments are lower than the performances theoretically resulting of the simple conjunction of the treatments administered alone at statistical level: α < 0.23 for 3 treatments. ▲Performances after association are smaller than the performances resulting theoretically of the simple conjunction of the treatments administered alone: Statistical level: α < 0.19 for 2 treatments; α < 0.23 for 3 treatments.
Plasmatic Hormonal Level Assays

Plasma adrenaline and noradrenaline levels were determined by a radioenzymatic method based on their conversion to their respective methoxylated derivatives by COMT addition to the incubation medium, in the presence of tritiated S-adenosyl methionine (picomolar sensitivity with a sample of 50 μl of plasma).

Corticosterone levels were measured by a radioenzymatic method (picomolar sensitivity with a sample of 500 nl of plasma).

Blood samples were taken from rats beheaded after the fifth conditioning series, after return to a normal atmospheric pressure for hypobaric hypoxic animals. Samples were then centrifugated (3000 g – 20 mn) and plasmas were frozen at –20°C (8 days) before assaying.

All the experiments were done between 9 and 11 a.m. to avoid the influence of circadian rhythms.

Statistical Analysis

All the results were analysed by Student’s t-test comparing treated rats to respectively normoxic controls. For behavioral studies, when two treatments (drug + hypoxia) or three treatments (drug + drug + hypoxia) were applied simultaneously, a potentialisation test was used.

Results

Behavioral Tests (tables 1 and 2)

Avoidance response was significantly lower in hypoxic control rats than in normoxic control rats (p < 0.01 days 1 to 5). No difference was observed for the total responses (avoidance + escape) between the two control series. Treatment with 1 mg/kg apomorphine induced a significant decrease of the total responses as compared to normoxic and hypoxic controls. However, apomorphine did not modify the avoidance responses of both normoxic and hypoxic rats during the 4 first days of test. On the fifth day, apomorphine induced an anti-hypoxic protection (table 1). The treatment with 0.5 mg/kg domperidone did not alter both the total and avoidance responses of either normoxic or hypoxic rats. Domperidone and apomorphine associated (the doses and delays were the same than when treatments were applied alone) did not modify the total response of normoxic or hypoxic rats. Domperidone therefore opposed the decrease in total performance caused by apomorphine both in normoxic and hypoxic rats; performances observed after association of the two drugs were not significantly different from control series. Apomorphine-induced anti-hypoxic effect was therefore left unchanged or increased by domperidone.

Adrenalectomy significantly decreased the avoidance response of hypoxic rats but we have not the results obtained for total responses. In all the cases, (normoxia and hypoxia) treatment with apomorphine of adrenalectomised rats induces an important fall of both total and avoidance responses.

Catecholamine and Corticosterone Levels (fig. 1)

Conditioned rats (5 days, at 760 torr) when com-
pared to naive rats (same handling but without conditioning) have significantly higher plasma levels of adrenaline \( (p < 0.01) \), noradrenaline \( (p < 0.01) \) and corticosterone \( (p < 0.05) \). Hypobaric hypoxia does not change the adrenaline or noradrenaline levels of conditioned rats, though there is a further increase in plasma corticosterone \( (p < 0.01) \) as compared to normoxic conditioned rats.

Pretreatment with apomorphine does not change the hormone levels in noroxic conditioned animals. In hypoxic conditions, pretreatment with apomorphine does not change the plasma adrenaline or noradrenaline levels, but increases the corticosterone levels. Analysis of variance however demonstrates an interaction between apomorphine and hypoxia for adrenaline levels \( (F_{16}^1 = 7.5, p < 0.025) \) and for corticosterone levels \( (F_{10}^1 = 4.35 \ p < 0.05) \) but not for noradrenaline levels \( (F_{10}^1 = 2.2) \).

Discussion

Avoidance and total responses are closely related: a pharmacological treatment preventing rats from climbing up the mast must obviously alter the avoidance responses; we cannot however tell whether this above treatment impairs the memorization ability. Thus we have to analyze the total and the avoidance responses.

In our experiments, hypobaric hypoxia does not alter total responses which are maximal from the second day on. However, the avoidance response is strongly altered. Thus we may say that it is the notion of learning itself which is directly impaired by hypobaric hypoxia.

Apomorphine \( (1 \text{ mg.kg}^{-1}) \) acts on both aspects of the rat's response: total responses are decreased in both normoxic and hypoxic animals. Nevertheless, it provokes an improvement of the avoidance responses in hypoxic rats already seen, in a larger extent for lower doses of apomorphine.\(^4\)

The decrease of total responses induced by apomorphine can be antagonized by domperidone whilst the "anti-hypoxic" protection is at the contrary reinforced. Since domperidone is known to not cross the blood brain barrier at least at the dose used\(^6\) we can

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**Figure 1.** Plasma adrenaline levels (A; pmoles \( \times \text{ml}^{-1} \)), plasma noradrenaline levels (NA; pmoles \( \times \text{ml}^{-1} \)) and plasma corticosterone levels (C; \( \mu g \times 100 \text{ ml}^{-1} \)) for naive rats (N), conditioned rats in normoxia (T) or hypoxia (TH) with or without pretreatment by apomorphine \( [1 \text{ mg} \times \text{kg}^{-1}] \) (A in normoxia; AH in hypobaric hypoxia). - mean \( \pm \text{SEM} \) in each case \( (n = \text{number of assays by group}) \). - Statistical analysis. 1) Student \( t \) test in reference with normoxic conditioned rats (T): \( \nabla: p < 0.05; \nabla\nabla: p < 0.01; \nabla\nabla\nabla: p < 0.005. \) Two-way analysis of variance showed a significant interaction between hypoxia and apomorphine for adrenaline \( (p < 0.025) \) and for corticosterone \( (p < 0.05) \) but not for noradrenaline levels.
infer that the decrease in total responses is a peripheral effect of apomorphine whilst the increase of tolerance of hypoxia is of central origin. These data confirm previous data showing for instance an antagonism of the "anti-hypoxic" effect of apomorphine by pimozide whose central effect is preeminent. In the same way the reinforcement of apomorphine induced anti-hypoxic protection by domperidone pretreatment can be related to the suppression of the nefarious effect of apomorphine on total performances.

The second point to analyse is the hypothesis of a protective effect of apomorphine mediated by an action on the adrenal glands: hypobaric hypoxia is a stress implicating the cortical and medullar adrenal metabolisms.

Our results show that adrenalectomy does not alter the avoidance responses of normoxic rats but depresses those of hypobaric hypoxic rats, showing the importance of the adrenal glands in conditions of severe experimental stress (learning + hypobaric hypoxia). Apomorphine pretreatment in adrenalectomized rats result in a decrease of total and avoidance responses in both normoxic and hypoxic rats. It remained to see whether the medullar or cortical adrenal plays the major role in these phenomena: plasma adrenaline and corticosterone reflect the variations of adrenal metabolism: 4/5ths of the body adrenaline come from the adrenal19,20 whilst plasma noradrenaline levels reflect the overall sympathetic activity.21,22

Adrenaline and Noradrenaline levels found in naive animals (handling without conditioning) are considerably higher than those usually reported in literature for chronically catheterized rats;23 beheading for blood samples can explain the difference.25,26 Conditioning increases the adrenaline, noradrenaline and corticosterone levels, presumably at least partly due to the experimental conditions (foot-shock). Moreover some authors27,28 have shown that the learning processes increase the sympathetic and adrenal activity with an increase in plasma adrenaline and noradrenaline levels when the conditioning tests are repeated.

There would therefore be an adaptative phenomenon, with an increase in the state of alertness of the animals as the days pass.26 Our results also agree with those reported in literature as to the increase of corticosterone levels during hypobaria,18 though we did not find the previously reported increases of adrenaline and noradrenaline levels.29 Hypobaria must however be considered as a stress,30 and this discrepancy can be explained by the fact that beheading and learning maximally increase adrenaline and noradrenaline levels,26 therefore masking the effects of hypobaric hypoxia.

Apomorphine (1 mg x kg⁻¹) does not change normoxic levels of adrenaline, noradrenaline and corticosterone. Analysis of the results in hypobaric hypoxia yields more data: Apomorphine interacts with hypoxia to decrease the adrenaline level and to increase the corticosterone level; Noradrenaline does not seem to be affected even though there also is a tendency to decrease.

This may be interpreted as a diminution of the adreno-medullar secretion and an increase of the adrenocortical secretion, both being related to a central stimulation of post-synaptic dopaminergic receptors.

Acknowledgments

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