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High sensitivity of brain octopamine levels to stress

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Abstract. Rats were submitted to unsignalled and uncontrolled electrical shocks. When re-exposed to the same situation but not shocked, 24 h later, their locomotor activity was significantly reduced compared to that of controls. This conditioned suppression was associated with a significant decrease in *p*-octopamine (OA) in brain stem and hypothalamus. Shocks delivered just before brain fixation produced an even larger decrease in cerebral OA. Heart levels of OA were not affected. Cerebral and peripheral levels of dopamine and noradrenaline were not significantly or reliably affected. These results, as those of previous experiments, suggest that octopamine is involved in emotional, neurovegetative responses to stress.

Key words: Octopamine – Catecholamines – Rat – Stress – Conditioned suppression

Octopamine (OA) was discovered in the posterior salivary gland of *Octopus Vulgaris*, about 30 years ago (Erspamer 1952). This amine exists in large amounts in the nervous system of invertebrates, in which it seems to be a neurotransmitter or neuromodulator (Batelle 1980; Dymond and Evans 1979; Evans and O'Shea 1977; Livingstone et al. 1981; Orchard and Loughton 1981; Robertson 1981). In vertebrates, the functions of OA have rarely been studied and are practically unknown, probably because of its very low concentration in the central nervous system (Buck et al. 1977; Danielson et al. 1977; Molinoff and Axelrod 1972; Talamo 1980). However, some functional correlations suggest that OA has a role in emotional and neurovegative responses: its production is deficient in patients suffering from depression (Sandler et al. 1979); its brain levels are higher in a hypertensive strain of rats (SHR Kyoto) than in a normotensive strain (David 1979); and brain levels of OA are lower in the Roman Low Avoidance (RLA) strain of rats than in the Roman High Avoidance strain (David and Delacour 1980). Moreover, administration of OA significantly increases active avoidance of unselected Wistar rats (David et al. 1982) and RLA (Delacour et al. 1983). Finally, OA administration decreases the suppressive effects of an inescapable stress on rat locomotor activity (Delacour and Guenaire 1983).

The experiments reported here tried to confirm and give further information on the above evidence by showing a direct connection between OA cerebral levels and emotional responses. A conditioned suppression technique was used for measuring an acquired emotional response. Immediately after the test of this response, the rat brain was fixed and amine levels measured. These measurements were then compared to those obtained in control rats and particularly in rats which did not acquire the emotional response.

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General methods

Animals

Fifty male Wistar rats weighing 200 g were used. During the entire experiment, they had free access to food and water. They were housed in individual cages and maintained on a 12:12 h light-dark cycle (0700–1900 hours). Ambient temperature was 23° C (±1).

Apparatus

The shuttle-box was a 60 × 30 × 50 (height) cm plexiglas cage divided into two compartements (30 × 30 cm) by a 5-cm barrier. The floor and the barrier were made of brass bars, 4 mm in diameter, and 15 mm apart. The crossing of the barrier was detected by an infrared system. Each compartment was equipped with a 15 W bulb fastened to the end wall, 35 cm above the floor. The apparatus was placed in a sound-insulated cabinet measuring approximately 1 m^3 in which sound insulation was aided by a masking white noise of 70 dBs above the human threshold. A 15 W bulb fastened to the ceiling of the cabinet provided constant illumination of 10 lux at the level of the floor of the shuttle-box. Programming and recording apparatus was located outside the cabinet and the animal was observed by means of closed-circuit television. Four identical shuttle-boxes located in four different cabinets permitted the simultaneous testing of four animals.

Neurochemical measurements

Immediately after the last session, the rats were quickly decapited, their brains frozen to −70° C and assayed within 1 week for OA as well as for dopamine (DA) and noradrenaline (NA). Octopamine was determined by using a modification of the methods previously described. Tissues were homogeneized in 5 vol of ice-cold 0.5 M Tris-HCl buffer [pH 8.6] containing 1 mM pargyline. Homogenates were

heated at 95° C for 3 min. After 5 min centrifugation at 1000 *g*, 150 µl aliquots of supernatant were incubated with 60 µl 0.05 M Tris-HCl (pH 8.6) containing 40 µl phenylethanolamine-N-methyltransferase (PNMT) solution and 0.04 nM (^{3}H)-S-adenosylmethionine [(^{3}H)-SAM; 13,5 Ci/nM, Saclay]. The reaction was stopped after 45 min by the addition of 200 µl 0.5 M borate buffer (pH 11), saturated with sodium chloride and containing 1 µg each of *p*-synephrine and N-methyl-phenylephrine. After extraction with ethyl-acetate and complete evaporation under nitrogen, the residues were dissolved in 1 ml saturated sodium carbonate and 0.5 ml acetone containing dansylchloride (8 mg/ml). They were allowed to react in the dark. The dansylated amines were separated with three successive and different TLC systems (Solvent I: chloroform-N-butylacetate 5:1 v/v; solvent II: toluene-triethylamine-methanol 50:5:1 v/v; solvent III: cyclohexane-ethyl-acetate 25:35, v/v). After the third separation, the spots revealed under UV light were scraped and their radioactivity was counted. Dopamine and noradrenaline were determined according to a previously described method (Robertson et al. 1977).

Statistical analysis

The number of barrier crossing in the shuttle-box on day 5 and the levels of OA, NA and DA of the different groups of rats were compared by means of a one-way variance analysis. When the overall F was significant ($P \leq 0.05$), two-by-two comparisons were made according to Winer (1971).

Experiment I

Methods

Behavioral testing was conducted for 5 consecutive days. The main behavioral measure was the number of barrier crossings in the shuttle-box taking place during a 24-min period on day 5. Shocks were pulsed, were 10 s long and had an intensity of 1 mA.

Rats from a homogeneous batch were randomly ascribed to five groups ($n=6$ each). Group 1 was tested in the shuttle-boxes during 4 consecutive days according to the following schedule: each day the rats were subjected to two sessions of 24 min in the morning and two sessions of 24 min in the afternoon. This group never received a shock. On day 5, rats were put into the shuttle-boxes for 48 min. The number of barrier crossings taking place during the last 24 min was measured.

The experiment began for the other groups on day 4; on this day, they were put in the shuttle-boxes for 12 min. Groups 2 and 3 received no shock; groups 4 and 5 received ten inescapable and unsignalled shocks. The interval between shocks varied from 40 to 90 s (mean 70 s). On the last day, day 5, group 3 received ten shocks under the same conditions as groups 4 and 5 on day 4. Groups 2, 4 and 5 were put in the shuttle-boxes for 24 min and did not receive shocks. For group 5 only, the experimental situation was modified: the white noise and the ambient illumination were suppressed in the shuttle-box.

The role of the different groups was as follows. Emotional response (suppression of locomotor activity) was principally measured by comparing group 4 (shocked on day 4) to group 2 (non-shocked). Group 5, shocked on day 4 and tested on day 5 in a modified experimental situation, served to test the specificity of the association between the experimental situation of day 4 and the shocks received in that situation. Group 3 permitted the evaluation of the immediate effects of electric shocks on amine levels. Group 1 was never shocked and was given a much longer habituation to the situation than the other groups. Rats were submitted to four 24-min sessions, on days 1–4 and on day 5, locomotor activity was measured during a 24-min period as for groups 2, 4 and 5, but this period was preceded by a 24-min habituation period. Due to this procedure, the animals in group 1, although never shocked, were assumed to have a low level of locomotor activity prior to brain fixation. Therefore, this group allowed the effects of electric chocks on amine levels to be dissociated from those of locomotor activity.

Results

Behavioral data. Table 1 gives the mean number of barrier crossings taking place during 24 min for the different groups on day 5, except for group 3 which was subjected to a different condition. Since the overall analysis of variance was significant ($F^3_{20}=14.51$, $P<0.001$), the groups were compared two by two. These comparisons show that:

1) The experimental procedure produced a significant suppressive effect on locomotor activity; group 4 (shocked on day 4) was significantly less active than group 2 (non-shocked).

2) Group 5 also displayed a suppressive effect on day 5, although tested in a situation different from day 4. The modification of the experimental situation was not sufficient to prevent generalization of the emotional response.

3) The locomotor activity of group 1 animals during the 24 min preceding brain fixation was significantly lower than that of group 2 and not significantly different from that of group 4; therefore, the neurochemical data of group 1 may serve to dissociate the effects of locomotor activity per se on the levels of the amines and the effects of emotional response.

Neurochemical data. The more significant results were obtained with cerebral *p*-OA (Table 2). Analysis of overall variance was significant in the brain stem ($F^4_{25}=191.86$, $P<0.001$) and hypothalamus ($F^4_{25}=103.65$, $P<0.001$). The two-by-two comparisons indicate that the shocks received by group 4 on day 4 induced a significant decrease in *p*-OA as compared to the measurements taken from group 2. Data from group 1 show that this effect is not the result of a change in locomotor activity, since group 1 showed a de-

Table 1. Conditioned suppression of locomotor activity (expt. I). Mean numbers of barrier crossings ± SEM taking place during 24 min. Before this test, group 1 was never shocked and was submitted to long habituation to the situation. Group 2 also was never shocked but was briefly habituated. Groups 4 and 5 received ten shocks the day before. Analysis of variance $F=14.51$, *df* 3, 20, $P<0.001$

Groups ($n=6$)	1	2	4	5
	9.16[a] ±5.9	65.0 ±8.37	19.33[a] ±5.67	15.66[a] ±6.45

[a] Significant difference from group 2 at 1% level

Table 2. Mean levels of *p*-octopamine in ng/g (experiment I). Before brain fixation, group 1 was never shocked and was submitted to long habituation to the situation; group 2 also was never shocked but was briefly habituated. Group 3 received ten shocks just before brain fixation. Groups 4 and 5 received ten shocks the day before. Analysis of variance $F=191.86$, df 4, 25, $P<0.001$ for brain stem, and $F=103.65$, df 4, 25, $P<0.001$ for hypothalamus

	Groups				
	1	2	3	4	5
Hypothalamus	5.95 ±0.33	5.65 ±0.24	0.90[a] ±0.06	2.81[a] ±0.21	2.33[a] ±0.1
Brain stem	1.69 ±0.05	1.58 ±0.08	0.35[b] ±0.03	0.89[b] ±0.05	0.83[b] ±0.03
Heart	32.21 ±1.78	33.38 ±1.92	28.33 ±1.62	31.25 ±1.74	33.83 ±1.22

[a] Significant differences from group 2 at 1% level
[b] Significant difference from group 2 at 1% level

Table 3. Mean levels of catecholamines in µg/g (experiment I). Before brain fixation, group 1 was never shocked and was submitted to long habituation to the situation; group 2 also was never shocked but was briefly habituated. Group 3 received ten shocks just before brain fixation. Groups 4 and 5 received ten shocks the day before. Analyse of variance $F=10.11$, df 4, 25, $P<0.01$ for NA hypothalamus

	Groups				
	1	2	3	4	5
NA Hypothalamus	1.33[a] ±0.03	1.46[a] ±0.05	1.04 ±0.02	1.39[a] ±0.05	1.29[a] ±0.06
NA Brain stem	0.37 ±0.03	0.32 ±0.03	0.28 ±0.02	0.34 ±0.02	0.29 ±0.02
NA Rest of brain	0.18 ±0.01	0.20 ±0.02	0.19 ±0.01	0.19 ±0.01	0.16 ±0.02
NA Heart	0.35 ±0.02	0.41 ±0.03	0.40 ±0.02	0.36 ±0.02	0.41 ±0.01
DA Hypothalamus	0.27 ±0.02	0.26 ±0.02	0.28 ±0.01	0.24 ±0.02	0.29 ±0.03
DA Brain stem	1.31 ±0.02	1.26 ±0.04	1.31 ±0.04	1.30 ±0.06	1.28 ±0.03
DA Rest of brain	0.52 ±0.04	0.51 ±0.02	0.44 ±0.05	0.36 ±0.03	0.46 ±0.04
DA Heart	1.43 ±0.05	1.39 ±0.04	1.51 ±0.03	1.54 ±0.04	1.56 ±0.03

[a] Significant difference at 1% level from group 3

crease in locomotor activity similar to group 4 and had normal levels of OA. Conversely, locomotor activity levels of groups 1 and 2 were significantly different (see behavioral data) but their OA levels were comparable. Thus the comparisons of groups 1, 2 and 4 from a behavioral and neurochemical standpoint show a double dissociation between OA levels and locomotor activity. The decrease in the level of OA was significant in group 5, where conditioned suppression was as pronounced as in group 4. In group 3, the immediate effect of ten inescapable and unsignalled shocks was a decrease in the level of cerebral OA that was

Table 4. Conditioned suppression of locomotor activity (expt. II). Mean numbers of barrier crossings ±SEM taking place during 24 min. Before this test, group 1 was never shocked and was submitted to long habituation to the situation. Group 2 also was never shocked but was briefly habituated. Group 4 received ten shocks and group 5, only one shock, the day before. Analysis of variance $F=23.57$, df 3, 12, $P<0.001$

Groups ($n=4$)	1	2	4	5
	10.75[a] ±1.63	59.0 ±2.67	7.5[a] ±3.3	20.25[a] ±8.77

[a] Significant difference from group 2 at 1% level

greater than that in groups 4 and 5. However, there was no significant difference between groups as to the level of OA in the heart.

In the case of catecholamines (Table 3), the only significant difference was obtained in the measurements of the hypothalamic level of NA ($F^4_{25}=10.11$, $P<0.01$). The two-by-two comparisons showed that group 3, which was shocked just before brain fixation, had a significant decrease in brain NA levels as compared to non-shocked groups (1 and 2) and groups 4 and 5 that were shocked 24 h before. Cerebral levels of NA were comparable in groups 1, 2, 4 and 5, which indicates that 24 h after their administration, the electric shocks had no effect on this amine.

Experiment II

Methods

As in experiment I, the behavioral tasks were conducted during 5 consecutive days and the main behavioral measurement was the number of crossings of the barrier taking place over 24 min on day 5. Rats from a homogeneous batch were randomly ascribed to five groups ($n=4$ each). Groups 1, 2, 3 and 4 were subjected to the same protocol as that used in experiment I. Group 5, on the contrary, was subjected to different treatment: (1) on day 4, group 5 of experiment II received only one shock whereas group 5 of experiment I received ten shocks. (2) On day 5, group 5 of experiment II was tested in exactly the same situation as on day 4, whereas group 5 of experiment I was tested in a different situation. Group 5 of this experiment was intended to test the sensitivity of cerebral OA levels to electric shocks received 24 h before. This is why this group received only one shock, whereas group 4 received ten shocks in experiments I and II.

As in experiment I, the behavioral dependent variable was the number of barrier crossings taking place during 24 min on day 5.

The neurochemical methods were the same as in experiment I.

Results

Behavioral data. Table 4 gives the mean number of barrier crossings for the different groups on day 5, except for group 3 which was subjected to a different condition. Since the analysis of overall variance was significant ($F^3_{12}=23.57$, $P<0.001$), the groups were compared two by two. These com-

Table 5. Mean levels of *p*-octopamine levels in ng/g (experiment II). Before brain fixation, group 1 was never shocked and was submitted to long habituation to the situation; group 2 also was never shocked but was briefly habituated. Group 3 received ten shocks just before brain fixation. Group 4 received ten shocks and group 5, only one shock, the day before. Analysis of variance $F=33.62$, *df* 4, 15, $P<0.001$ for hypothalamus and $F=81.1$, *df* 4, 15, $P<0.001$ for brain stem

	Groups				
	1	2	3	4	5
Hypothalamus	5.25 ±0.49	5.93 ±0.55	0.87[a] ±0.05	2.17[a] ±0.12	6.13 ±0.45
Brain stem	1.71 ±0.07	1.6 ±0.11	0.28[a] ±0.02	0.93[a] ±0.04	1.65 ±0.05
Heart	26.15 ±4.06	27.88 ±2.18	25.58 ±1.29	31.5 ±0.64	32.2 ±1.91

[a] Significant difference from group 2 at 1% level

Table 6. Mean levels of catecholamines in μg/g (experiment II). Before brain fixation, group 1 was never shocked and was submitted to long habituation ot the situation; group 2 also was never shocked but was briefly habituated. Group 3 received ten shocks just before brain fixation. Group 4 received ten shocks and group 5, only one shock the day before. Analysis of variance $F=3.08$, *df* 4, 15, $P<0.05$ for DA hypothalamus

	Groups				
	1	2	3	4	5
NA Hypothalamus	1.31 ±0.05	1.34 ±0.06	1.44 ±0.05	1.4 ±0.07	1.38 ±0.07
NA Brain stem	0.46 ±0.03	0.45 ±0.02	0.53 ±0.04	0.43 ±0.03	0.47 ±0.05
NA Rest of brain	0.24 ±0.02	0.24 ±0.01	0.27 ±0.04	0.23 ±0.02	0.22 ±0.01
NA Heart	0.35 ±0.03	0.38 ±0.01	0.33 ±0.02	0.35 ±0.05	0.34 ±0.03
DA Hypothalamus	0.29[a] ±0.02	0.31[a] ±0.02	0.35 ±0.03	0.39 ±0.03	0.29[a] ±0.01
DA Brain stem	1.19 ±0.05	1.26 ±0.02	1.25 ±0.04	1.19 ±0.03	1.21 ±0.04
DA Rest of brain	0.45 ±0.03	0.44 ±0.04	0.41 ±0.05	0.41 ±0.02	0.42 ±0.05
DA Heart	1.36 ±0.06	1.36 ±0.03	1.43 ±0.07	1.44 ±0.05	1.41 ±0.05

[a] Significant difference from group 4 at 5% level

parisons confirm the main results of experiment I: Group 4 shocked on day 4 was significantly less active than group 2. Group 1, habituated for a long time to the experimental situation, showed reduced locomotor activity when compared to group 2, but did not differ significantly from group 4. Therefore, as in experiment I, this group may serve to dissociate the effects on amine brain levels of emotional response from that of the locomotor activity displayed just before brain fixation. These comparisons also showed that one shock was sufficient to induce a suppressive effect on locomotor activity, since the latter was significantly lower in group 5 when compared to group 2. This reduction was less important than in group 4 which received ten shocks; however, the difference between groups 4 and 5 approached ($P<0.1$) but did not reach the significance threshold ($P=0.05$).

Neurochemical data. The main results of experiment I were confirmed. Brain stem and hypothalamic levels of OA were significantly lower in group 4 than in group 2. This effect was not simply due to a decrease in locomotor activity, since group 1 showed a normal level of OA. In rats shocked just before brain fixation, the level of OA was more reduced than in group 4 (Table 5). The most important result was that group 5 did not show a significant decrease in cerebral OA level, even though its locomotor activity was significantly reduced.

As in experiment I, catecholamine levels were in most cases unsensitive to the different treatments (Table 6). One difference, however, should be stressed,: in experiment I, hypothalamic NA levels showed a significant decrease in group 3, whereas DA levels were not affected in any group. In experiment II, hypothalamic NA levels were not affected, but the hypothalamic DA level was significantly increased in group 4.

Discussion

The above experiments principally show that the cerebral levels of OA are highly sensitive to stress. The level of this amine was decreased in the hypothalamus as well as in the brain stem of rats shocked either just before brain fixation or 24 h before. This effect did not depend on locomotor activity, as shown by the comparisons of groups 1, 2 and 4, and is probably not the consequence of peripheral modifications, since the measurements of OA levels in the heart were comparable in all groups.

On the other hand, catecholamine levels were less sensitive to stress. In experiment I, DA levels were not affected and NA levels were significantly reduced only in the hypothalamus of recently shocked rats. These results agree with other published data: stresses more intense than those used in our experiments did not affect DA levels (Herman et al. 1982; Weiss et al. 1981). The NA levels are more sensitive but only when the shocks are administered immediately or shortly before brain fixation. In only one study (Weiss et al. 1980) was it reported that NA levels were reduced 24 h after stress, but this stress was much more severe than that used in our experiments. In experiment II, shocks delivered 24 h prior to brain fixation significantly increased the level of hypothalamic DA. This unexpected finding cannot yet be explained. A control neurochemical experiment confirmed the results of experiment I: 24 h after administration of ten shocks, no modification of NA and DA levels could be observed in the central nervous system or in peripheral tissues. In contrast, ten shocks administered just before brain fixation elicited a significant decrease in hypothalamic NA level.

The relative unsensitivity of catecholamine levels does not mean that the role of these amines in responses to stress is negligible. Perhaps their level is not the adequate variable. Other measurements, such a those of the metabolites of NA and DA (Cassens et al. 1981; Herman et al. 1982; Tanaka et al. 1982) or the rate of turnover (Lane et al. 1982), may be more sensitive.

The meaning of the correlations between behavioral and neurochemical effects of stress is generally problematic. Are changes in the level or turnover of a neurotransmitter a simple persistence of the effect of the stress, or rather a "conditioned" response elicited by re-exposure to the stress situation? An increase in DOPAC in the frontal cortex has been observed in rats re-exposed to a stress situation 24 h after the stress (Herman et al. 1982). This increase was not present after the same delay in stressed rats which had not been reexposed to the situation where the stress was delivered but stayed in their home cages. This suggests that the DOPAC increase could be "conditioned"; however, the reexposed and the non-reexposed groups were subjected, just before brain fixation, to quite different conditions with respect to locomotor activity, sensory stimulations, arousal state, etc. Consequently, the difference in DOPAC level is difficult to interpret.

In experiment I, we attempted to check the conditioned character of the decrease in OA by exposing one group of rats before brain fixation (group 5) to a situation different but comparable to the stress situation. Unfortunately, rats in this group showed a gneralization of their emotional response: their locomotor activity and their cerebral OA levels were decreased in the same proportion as in group 4. Consequently, either the "persistence" or the "conditioning" hypothesis may account for the OA decrease in group 4. The behavior of group 5 in experiment II showed that conditioned suppression of locomotor activity could be observed without a concomitant change in OA levels. This result favors the persistence hypothesis – it is likely that the effect of one shock (the stress applied to group 5) persists less than that of ten shocks (the stress applied to group 4).

Whatever that may be, our results suggest that OA plays an important role in responses to stress. The exact nature of this role remains to be elucidated, particularly in relation to that of NA. OA may be a neuromodulator of the noradrenergic system and as such, play a role in depression and/or in antidepressant treatments (Edwards 1982; Harmar 1980). Another possibility is that the changes in OA levels merely are a reflection of changes in the turnover of NA. The turnover of OA is much higher than that of catecholamines (Talamo 1980) and a small change in NA output could lead to much larger increases in OA output. This hypothesis will be tested in further experiments.

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