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Yawning and Suppression of Exploration Induced by Dopamine Agonists: No Relation to Extracellular Striatal Levels of Dopamine

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STÅHLE, L. AND U. UNGERSTEDT. *Yawning and suppression of exploration induced by dopamine agonists: No relation to extracellular striatal levels of dopamine.* PHARMACOL BIOCHEM BEHAV **35**(1) 201–209, 1990.—The present study was aimed at testing the hypothesis that yawning and suppression of exploration induced by low doses of dopamine agonists in the rat are caused by a reduction of synaptic dopamine levels. The decrease in extracellular levels of dopamine in the corpus striatum induced by α-methyl-p-tyrosine (αMPT, 50–200 mg/kg IP), reserpine (2–5 mg/kg SC) and apomorphine (APO, 0.05 mg/kg SC) was measured in microdialysis experiments. Reserpine and αMPT reduced the dopamine levels to the same extent as APO. Exploratory behaviour was suppressed by APO, but not by αMPT (50 and 100 mg/kg) when tested in a separate experiment. Reserpine (2 mg/kg) suppressed exploration after 4 hr, but not after 3 hr. Changes in extracellular levels of dopamine were tested simultaneously with changes in yawning in another group of rats implanted with guide cannulae for microdialysis probes. There was a discrepancy in the time-course for the induction of yawning as compared to the changes in extracellular dopamine levels after APO (0.05 mg/kg) as well as after pergolide (0.02 mg/kg SC). Yawning appeared before and lasted shorter than the decrease in dopamine. The time-courses for APO-induced suppression of exploration and yawning were similar. The dose-response curve for APO-induced yawning was not changed by αMPT (200 mg/kg), while the suppression of exploration induced by APO, but not by pergolide, was enhanced by pretreatment with αMPT. The results show that yawning and suppression of exploration induced by dopamine agonists are not related to changes in extracellular levels of dopamine. It is proposed that these behaviours may be mediated by postsynaptic receptors.

Apomorphine Autoreceptor Dopamine Exploration α-Methyl-dl-p-tyrosine Microdialysis Pergolide Reserpine Yawning

DOPAMINE (DA) autoreceptors (14) are believed to autoregulate the activity of DA neurons by inhibiting release of DA (5, 20, 56, 69, 77), inhibiting tyrosine hydroxylase (26, 59, 67), decreasing DA utilisation (4) and reducing the firing of DA neurons (1, 2, 44, 68). The autoreceptors are assumed to be more sensitive to treatment with DA agonists than the postsynaptic receptors (44,59). It has also been hypothesised that behavioural effects induced by low doses of DA agonists are mediated by stimulation of DA autoreceptors (10, 12, 15, 17, 18, 22, 28, 31, 59, 60, 74). Thus, the behavioural effects of DA agonists such as yawning and motor inhibition have been used to screen for autoreceptor active substances (7, 12, 23, 46, 70). However, it has been suggested that DA agonist-induced yawning (37, 41, 42, 50, 51), sedation (41) and suppression of exploratory behaviour (49,51) are not mediated by stimulation of DA autoreceptors.

The present study was undertaken to further investigate the hypothesis that the behavioural effects of low doses of DA agonists are mediated by a reduction of the extracellular levels of DA (from hereon referred to as the autoreceptor hypothesis). This was done by comparing the effects on extracellular levels of DA of apomorphine (APO), the tyrosine hydroxylase inhibitor α-methyl-dl-p-tyrosine (αMPT) and the monoamine storage blocker reserpine. Microdialysis (64) was used to sample the extracellular DA. The results from the microdialysis experiments were then compared to behavioural effects of the drugs. The findings suggest that the behavioural effects of low doses of DA agonists are *not* correlated to the extracellular levels of DA. Preliminary accounts of the present work have been published elsewhere (45,49).

METHOD

Subjects

Male Sprague-Dawley rats (ALAB, Sollentuna, Sweden) weighing 140–240 g were used throughout. They were delivered to the animal department at least two days before the experiment and were housed five per cage. The animals had access to ordinary lab chow and tap water (ad lib) except during experimental procedures. One hour before behavioural experiments the animals were

transferred to the laboratory and put in individual cages. They were maintained on a 12-hr light/dark cycle. Each animal was used once.

Stereotaxic Surgery and Microdialysis

Anaesthesia was induced and maintained by halothane using a constant airflow of 1 l/min. In order to compensate for loss of body fluid during the experiment, 2.5 ml Ringer solution were injected every 2 hr SC in the neck. Body temperature was kept constant at 36.6–37.3°C. The rats were placed in a David Kopf stereotaxic instrument with the incisor bar at −2.4 mm. A microdialysis probe (CMA/10, Carnegie Medicin AB, Stockholm, Sweden) with 3.0×0.5 mm (length × outer diameter) diffusible area was implanted unilaterally into the striatum (stereotaxic coordinates: 1.3 anterior to bregma, 2.2 mm lateral to the midline and 6.6 mm ventral to the brain surface). Intracerebral guide cannulae (Carnegie Medicin AB), which allow the dialysis probe to be inserted when the rat is awake, were implanted at least two days prior to the experiment (coordinates with reference points as above: +1.3, 2.2, 3.0) and only the 3 mm diffusible part of the probe extended beyond the tip of the guide cannula. A degassed Ringer solution (ACO, Sweden) was perfused through the dialysis probe at a constant flow-rate of 2 μl/min and was collected in 10-min (20 μl) or 20-min (40 μl) fractions. To prevent breakdown of DA, 10 μl of 1.0 M perchloric acid were added to each fraction. Drugs were injected when a stable baseline of DA was attained, usually about 2 hours after the start of the perfusion. DA was measured by electrochemical detection after separation on a 3.0 mm C18 column with a mobile phase consisting of .0.15 M phosphate buffer, 0.8 mM octanesulfonic acid, 0.1 mM EDTA, 13.8% methanol, pH 3.80 [for details see (43)].

Yawning Behaviour

Yawning was recorded immediately after injection of the drug for 40–60 min in 10-min periods by direct observation. The animals were placed in individual cages at least one hour prior to the observations.

Holeboard Behaviour

A holeboard apparatus (70×70 cm) with 32 holes, equipped with infrared photocells, was used as described in detail elsewhere (29,47). The ten behavioural variables recorded were: activity, forward locomotion, total locomotion, hole count, hole time, corner count, corner time, rearing count, rearing time and habituation.

Statistics

Yawning data were presented as means and hypothesis testing was made by Kruskal-Wallis one-way ANOVA and Mann-Whitney U-test. Holeboard and microdialysis data were analysed by a multivariate statistical method called partial least squares analysis (PLS) as described in detail elsewhere (53–55, 71, 73). PLS can be used in a multiple-regression-like way to evaluate dose-response relationships (53). However, instead of regression on all response variables, a score is formed from the originally measured variables (e.g., the holeboard measurements). The score is a weighted sum of the measured variables. In some cases PLS was used as a multivariate analogue to ANOVA (54). The PLS *t*-score in the holeboard experiments may be interpreted as a measure of exploratory behaviour. For those familiar with PLS, some details of its use here are given below. For general introductions to PLS, interested readers are referred to review articles (53,55).

The observed data from a complete experiment are organised in a table with one row for each rat and one column for each behavioural or neurochemical variable. Data are normalised to zero mean and unit variance variable-wise. This table is denoted X. In another table, Y, the columns are the log-dose (with controls one log-step below the lowest dose in order to use as much information as possible). In an ANOVA-like analysis, Y consists of 0–1 dummy variables (as many as there are groups) that denote group membership [see (54)]. The rows are exactly the same as in X. The relation between the recorded data (X) and dose (or group membership) (Y) is calculated by PLS via the *t*-scores (latent variable in X), the u-scores (latent variable in Y) and the inner relation d which is the regression coefficient between *t* and u. The *t*-scores are calculated from weight coefficients (w_k for the k:th holeboard variable or the k:th collected microdialysis fraction).

$$t_i = \sum_{k=1}^{p} w_k x_{ik}$$

where x_{ik} is the recorded value for the k:th holeboard variable (k=1..p) for the i:th rat. Similarly, there are weight coefficients b_j (j=1..q) for the calculation of u-scores. The significance of a dose-response relation or a ANOVA-like analysis is assessed by cross-validation (72,73) where a significant (at the 5% level) cross-validation deviation/standard deviation ratio (cvd/sd) shall be below a critical level (54). The significance of the weights was assessed by constructing 95% confidence intervals using the jackknife estimate of variance (9, 35, 73). Missing values were assessed by principal components analysis to improve the estimation of the shape of time-response curves (Ståhle, in preparation). The results are presented as mean ± SEM *t*-scores, weights for X (w) and Y (b) and cvd/sd. Neurochemical data are presented as the observed variables, but were analysed by the corresponding PLS analyses.

Drugs

Apomorphine-HCl (APO) was dissolved in saline by rapid heating and injected subcutaneously (SC) in the flank in a volume of 1 ml/kg. αMPT was dissolved in saline and injected intraperitoneally (IP) in a volume of 2 ml/kg. Reserpine was injected SC as a commercially available solution (Serpasil®). Pergolide was dissolved in distilled water and diluted in saline and injected as APO.

Experiments

The effects of the following treatments were studied:

1. αMPT (50 mg/kg, n=7; 100 mg/kg, n=6; 200 mg/kg, n=6) on extracellular levels of DA in anaesthetised rats. Samples were taken every 20 min.
2. Reserpine (2 mg/kg, n=7; 5 mg/kg, n=6) on extracellular levels of DA in anaesthetised rats. Samples were taken every 20 min.
3. APO (0.05 mg/kg, n=7) or pergolide (0.005 mg/kg, n=6; 0.02 mg/kg, n=7) on extracellular levels of DA and yawning behaviour in conscious rats implanted with guide cannulae for the microdialysis probe. Samples were taken every 10 min in the APO experiment and every 20 min in the pergolide experiments.
4. αMPT (200 mg/kg 4 hr before injection of APO) or reserpine (10 mg/kg 18 hr before APO) plus αMPT (200 mg/kg 4 hr before APO) on the dose-response curve (APO 0.01–0.1 mg/kg) of APO-induced yawning (n=5–9). Yawning was counted for 40 min.

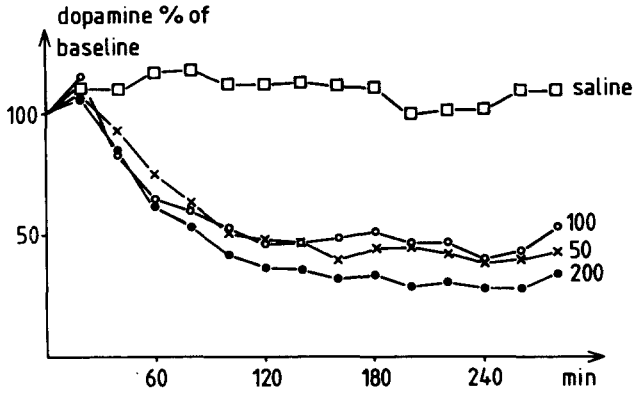

FIG. 1. Dose- and time-response to αMPT (0–200 mg/kg) on extracellular levels of dopamine. SEM = 3–14%. Basal dopamine levels were saline 7.0 ± 1.0 nM, 50 mg/kg 6.9 ± 1.2 nM, 100 mg/kg 6.9 ± 1.7 nM and 200 mg/kg 4.7 ± 0.5 nM.

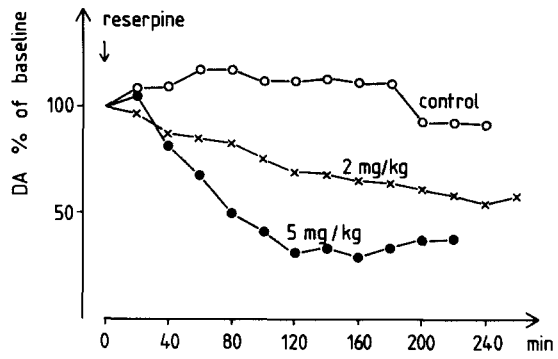

FIG. 2. Dose- and time-response to reserpine (0–5 mg/kg) on extracellular levels of dopamine. SEM = 5–14%. Basal dopamine levels were saline 7.0 ± 1.0 nM, 2 mg/kg 10.6 ± 1.2 nM and 5 mg/kg 11.0 ± 4.7 nM.

5. Time-response to APO (0.05 mg/kg) on suppression of exploratory behaviour recorded for 10 min in the holeboard. Pretreatment times were 0 min (n = 8), 15 min (n = 11), 35 min (n = 7), 55 min (n = 7) and 75 min (n = 7).
6. Dose- and time-response to αMPT (controls, n = 8; 50 mg/kg, n = 6; 100 mg/kg, n = 6; 200 mg/kg, n = 6) on suppression of exploration. The pretreatment times in the time response to αMPT (200 mg/kg) were controls (n = 10), 20 min (n = 6), 60 min (n = 6), 120 min (n = 5) and 240 min (n = 6).
7. Dose-response to reserpine with 3-hr pretreatment time (controls, n = 6; 1 mg/kg, n = 4; 2 mg/kg, n = 6; 5 mg/kg, n = 6) or 4-hr pretreatment time (controls n = 4; 1 mg/kg, n = 4; 2 mg/kg, n = 6; 5 mg/kg, n = 6) on exploration.
8. Interaction between αMPT (200 mg/kg 4 hr before testing) and APO (0.05 mg/kg 15 min before testing) or pergolide (0.02 mg/kg 15 min before testing) on suppression of exploration (n = 6–8).

RESULTS

Effect of αMPT on Extracellular Levels of Dopamine

All doses of αMPT reduced the DA levels substantially (Fig. 1). A dose of 50 or 100 mg/kg reduced the DA levels to 40% of baseline values. The highest dose of αMPT (200 mg/kg) caused a maximum reduction of DA levels to 25% of baseline. The effect of αMPT was apparent in the fraction 20–40 min after injection. There was no tendency for the effect to wear off during the 4-hr 40-min postinjection period.

Effect of Reserpine on Extracellular Levels of Dopamine

Doses of reserpine below 2 mg/kg had no effect on the extracellular levels of DA (data not shown). The effect of 2 mg/kg was apparent after 80 min and the levels declined to 60% of baseline in the dialysis fraction collected 180–200 min after injection and continued to decrease to approximately 55% after 4 hr (Fig. 2). The effect of 5 mg/kg reserpine was more rapid in onset and also more pronounced. After 2 hr 40 min, the maximum effect was attained and the DA levels remained stable at 35% of baseline.

Effects of APO and Pergolide on Extracellular Dopamine Levels and Yawning

In awake rats the change in extracellular levels of DA and the appearance of yawning were monitored simultaneously. The sampling period for dialysates was shortened to 10 min in the APO (0.05 mg/kg) experiment. The levels of DA were reduced to 55% of baseline with the maximum effect after 30–40 min postinjection (Fig. 3a). The DA levels were back to normal 120 min after administration of APO. Yawning appeared shortly after injection and the maximum effect was within the first 10 min (Fig. 3b). No yawning was observed 40 min after injection. Pergolide 0.005 mg/kg had no effect on extracellular DA levels and induced yawning only to a small extent (Fig. 3c). A larger dose of pergolide (0.02 mg/kg) reduced the extracellular DA levels to 40% of baseline and induced a significant amount of yawning (Fig. 3d). The peak effects were after 20–40 min for yawning and 40–100 min for DA levels.

Interaction Between αMPT, Reserpine and APO on Yawning

There was a dose-dependent effect of APO (0.01–0.1 mg/kg) on the appearance of yawning behaviour (Fig. 4). Pretreatment with αMPT (200 mg/kg) alone did not affect the dose-response curve of APO-induced yawning (Fig. 4). When both αMPT (200 mg/kg) and reserpine (10 mg/kg) were given before APO the yawning response was partially inhibited.

Time-Response to APO on Exploration

The effect of APO (0.05 mg/kg) was almost maximal 0–10 min after injection and only slightly more pronounced after 15–25 min (Fig. 5). After 35–45 min or more the effect of APO was nonsignificant. Variable weights are given in Table 1.

Dose- and Time-Response to αMPT on Exploration

There was a dose-dependent suppression of exploration by αMPT given 4 hr before testing. The lowest doses (50–100 mg/kg) had no effect on behaviour, while 200 mg/kg significantly suppressed exploration (Fig. 6a). The time course of the effect of αMPT (200 mg/kg) is shown in Fig. 6b. The effect of αMPT is significant 2 hr after injection and maximal at 4 hr. Later time-points were not tested. Variable weights are given in Table 1.

Dose-Response to Reserpine on Exploration

The lowest doses (1–2 mg/kg) had no effect on exploration 3 hr after injection, but 2 mg/kg suppressed exploration 4 hr after injection (Fig. 7). However, 5 mg/kg caused a maximal response

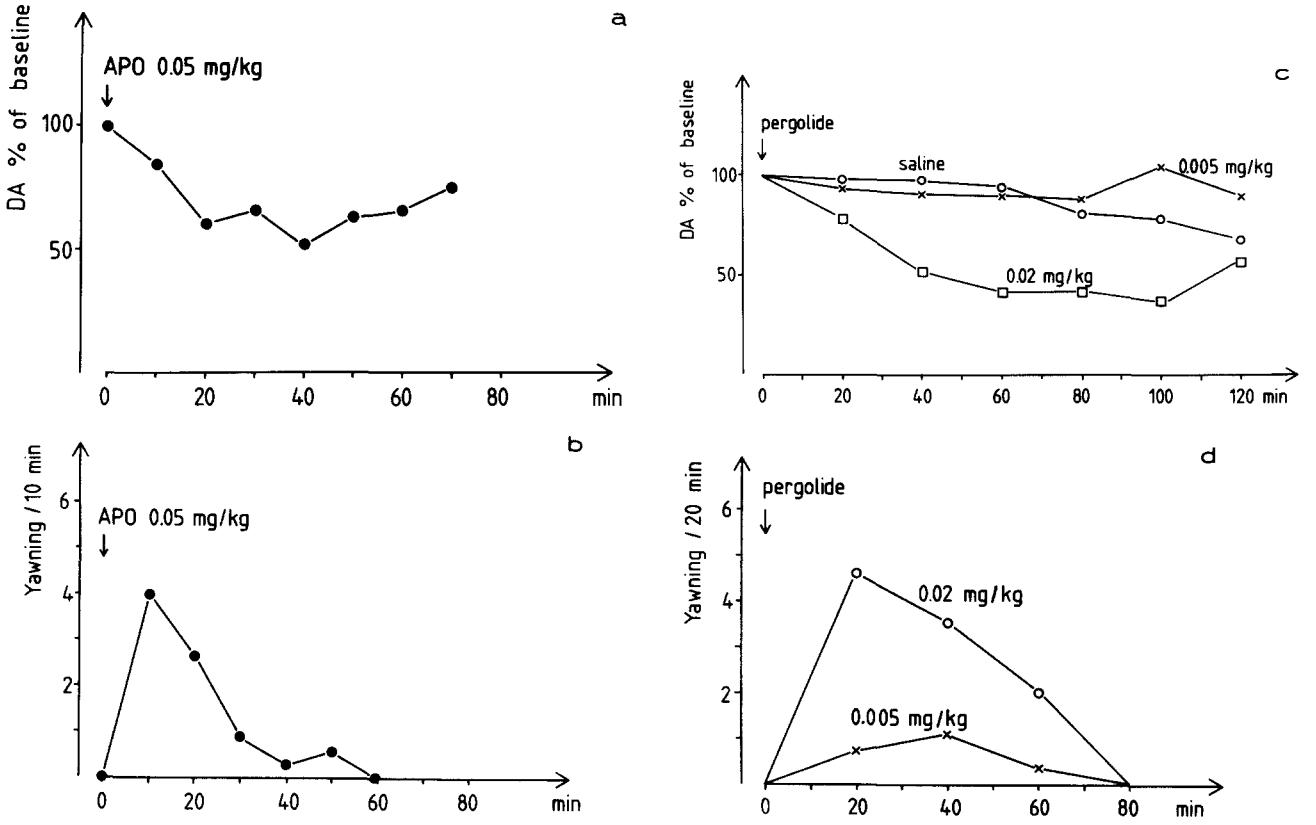

FIG. 3. Yawning behaviour and extracellular levels of dopamine monitored simultaneously. The graphs illustrate the effects of APO (0.05 mg/kg) on a) dopamine and b) yawning (both measured during 10-min periods) and the effects of pergolide (0.005 and 0.02 mg/kg) on c) dopamine and d) yawning (20-min periods). For dopamine SEM = 3–11%. Basal dopamine levels were saline 3.0 ± 0.9 nM, APO 0.05 mg/kg 4.0 ± 1.2 nM, pergolide 0.005 mg/kg 3.7 ± 1.2 nM and 0.02 mg/kg nM.

at both time-points. Rats treated with 5 mg/kg were almost completely inactive (compare the effect of reserpine 5 mg/kg with the standard error bar of the control animals in this and the αMPT experiment) and these animals also displayed pronounced catalepsia (data not shown). Variable weights are given in Table 1.

Interaction Between αMPT and APO or Pergolide on Exploration

The suppressive effects of APO (0.05 mg/kg) and αMPT (200 mg/kg) were approximately equal, while the effect of pergolide (0.02 mg/kg) was more pronounced (Fig. 8). When APO or pergolide were given together with αMPT (200 mg/kg) there was an addition of the suppressive effects on exploration of αMPT and APO, but not of αMPT and pergolide (Fig. 8).

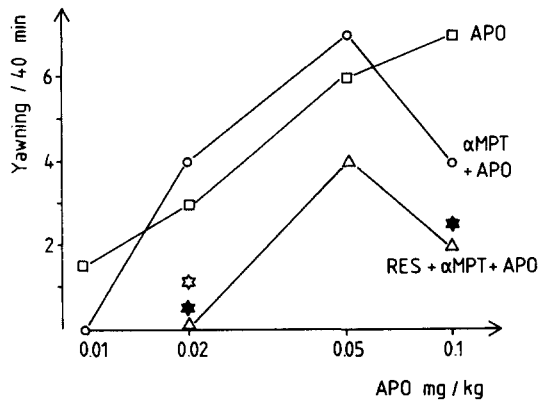

FIG. 4. Dose-response curves for APO on yawning in rats pretreated with saline, αMPT (200 mg/kg) or αMPT (200 mg/kg) + reserpine (10 mg/kg). Statistics: APO 0.01, U = 9, NS; APO 0.02, H = 7.5, $p<0.05$; APO 0.05, H = 1.2, NS; APO 0.1 H = 6.9, $p<0.05$.

DISCUSSION

The idea that low doses of DA agonists selectively stimulate

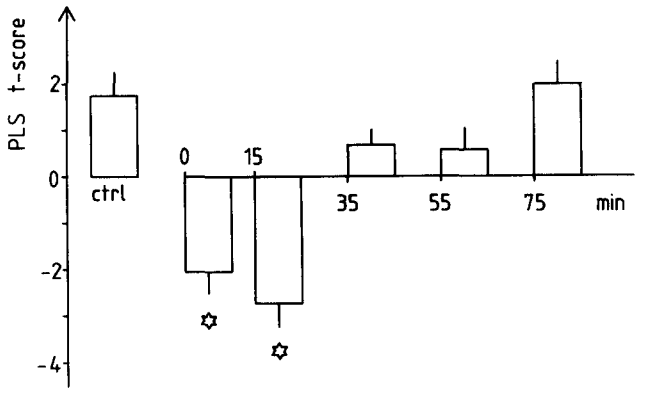

FIG. 5. Time-response to APO (0.05 mg/kg) on suppression of exploration. The behaviour is measured as a PLS *t*-score (see the Method section) which may be interpreted as a measure of exploratory activity. The ANOVA-like PLS methodology was used to analyse the data. The zero-level is the overall mean and the scale is in standard deviations.

TABLE 1

WEIGHTS FOR THE RECORDED HOLEBOARD VARIABLES USED TO CALCULATE THE *t*-SCORES IN THE EXPERIMENTS INDICATED IN THE TOP ROW

	αMPT Dose-Response	αMPT Time-Response	Reserpine Dose-Response	αMPT, APO Pergolide Interaction	APO Time-Response
ACT	0.484*	0.408*	0.407*	0.389*	0.416*
FL	0.353*	0.377*	0.391*	0.355*	0.409*
TL	0.423*	0.391*	0.375*	0.354*	0.409*
HC	0.431*	0.418*	0.379*	0.374*	0.343*
HT	0.141	0.150	0.355*	0.319*	0.292*
CC	0.182	0.272*	0.255*	0.267*	0.343*
CT	0.043	0.080	0.028*	−0.114	−0.102
RC	0.047	0.146	0.328*	0.283*	0.272*
RT	0.042	0.063	0.310*	0.294*	0.241*
HAB	0.430*	0.440*	0.031	0.319*	0.132
cvd/sd	0.785*	0.955*	0.928*	0.934*	0.937*

Statistically significant weights at the 5% level, according to jackknife estimates of the variances of the weights, are indicated by *. The first column is the names of the variables abbreviated: ACT—activity, FL—forward locomotion, TL—total locomotion, HC—hole count, HT—hole time, CC—corner count, CT—corner time, RC—rearing count, RT—rearing time, HAB—habituation and cvd/sd is the cross-validation test statistic described in the Method section.

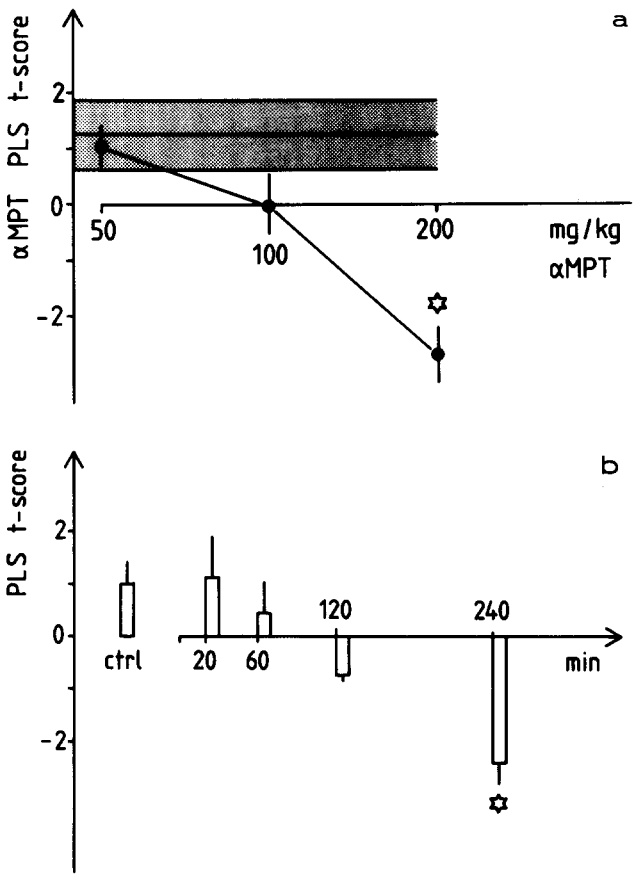

FIG. 6. (a) Dose-response curve for the effects of αMPT (0–200 mg/kg) given 4 hr before testing for suppression of exploration in the holeboard and (b) the time-response to αMPT 200 mg/kg. The measurement is the PLS *t*-score as described in the Method section. The *t*-score may be interpreted as a measure of exploratory activity. Log dose was used as the dependent variable (Y) in the dose-response analysis and the ANOVA-like PLS methodology was used to analyse the time-response data. The zero-level represents the overall mean in the experiment and the scale is in standard deviations.

DA autoreceptors was originally put forward by Carlsson (14) and Strömbom (59) and also by Ljungberg and Ungerstedt (28) and DiChiara *et al.* (17). The hypothesis is based on the finding that low doses of DA agonists inhibit tyrosine hydroxylase (14,59) without inducing stereotyped behaviour (28,59) which has been subsequently substantiated (10, 12, 15, 60). The fact that APO in the low-dose range also suppresses exploration (28,59), and that the effects of a wide range of DA agonists on behaviour and synthesis are strongly correlated (12), suggested that suppression of exploration also is mediated by DA autoreceptors. Similar arguments were used to formulate the same hypothesis for yawning behaviour (22, 36, 74).

Other evidence supporting the autoreceptor hypothesis has been put forward. Yawning behaviour can be suppressed by pretreatment (4 hours) with reserpine (36). This was interpreted as a maximal reduction of synaptic DA to which the effect of APO could not be added. Surprisingly, Yamada and Furukawa (74) found that reserpine (24 hours pretreatment time) enhanced yawning which they interpreted as an additive effect of APO and reserpine. In both cases the findings were taken as evidence in favour of the autoreceptor hypothesis. Serra *et al.* (42) reported that reserpine alone induces yawning at 24-hr pretreatment, but not at 4 hr, which they interpreted as an argument against the autoreceptor hypothesis.

Dourish and Hutson (19) found that 6-OHDA lesions of striatal DA terminals abolish yawning behaviour induced by APO 0.1 mg/kg. The authors interpreted their findings as due to a loss of autoreceptors. Similarly, recent observations by Stoessel *et al.* (58) show that even smaller doses of APO did not induce yawning in rats with 6-OHDA-induced substantia nigra lesions as would have been expected if postsynaptic receptors had mediated the response. However, intrastriatal injections of DA agonists elicit yawning in rats with local 6-OHDA lesions at the injection site (41). Hence, it cannot be excluded that the results of Dourish and Hutson (19) and Stoessel *et al.* (58) are caused by nonspecific blockade of the expression of yawning, e.g., through behavioural competition. Recently, Melis *et al.* (33) suggested that yawning is mediated by hypothalamic DA.

The present study was undertaken to further test the hypothesis that suppression of exploration and induction of yawning by low doses of DA agonists are mediated by stimulation of DA autoreceptors. From the autoreceptor hypothesis a number of predictions follow. In the present study three such predictions were tested:

1. The relation between the synaptic levels of DA and behaviour should be the same if DA levels are reduced by an agonist or by some other drug, e.g., αMPT (a tyrosine hydroxylase inhibitor) or reserpine (a monoamine storage blocker).
2. The reduction of synaptic levels of DA and the behavioural effects of DA agonists should have a similar time-course.
3. The dose-response curve for the behavioural effects of a DA agonist should be shifted to the left (i.e., the response should be enhanced) by pretreatment with αMPT.

Methodological Considerations

This study uses the microdialysis method to monitor extracellular levels of DA. It is, therefore, necessary to discuss to what

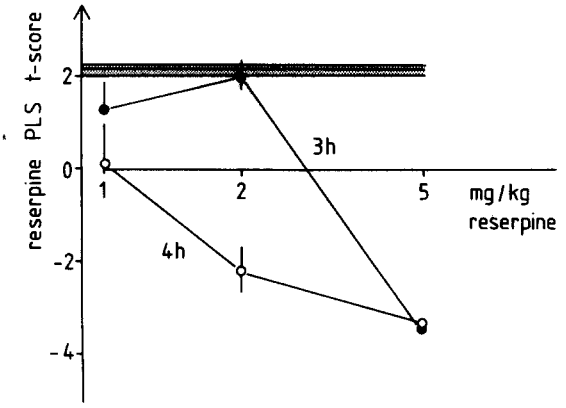

FIG. 7. Dose-response to reserpine (0–5 mg/kg) given 3 or 4 hr before testing for suppression of exploration in the holeboard. The measurement is the PLS *t*-score using log dose as the dependent variable (Y). The *t*-score may be interpreted as a measure of exploratory activity. The zero level is the overall mean and the scale is in standard deviations.

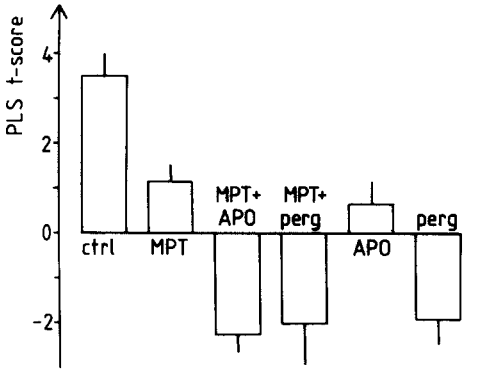

FIG. 8. Effects on holeboard behaviour of αMPT (200 mg/kg), APO (0.05 mg/kg) and pergolide (0.02 mg/kg) given alone or in combination (except APO plus pergolide) compared with saline-treated controls. The behaviour is measured as a PLS *t*-score (see the Method section) which may be interpreted as a measure of exploratory activity. The ANOVA-like PLS methodology was used to analyse the data. The zero-level is the overall mean and the scale is in standard deviations.

extent sampling of the extracellular space by the microdialysis method faithfully mirrors the events in the synaptic cleft. There is, unfortunately, no method available to investigate this problem in a straightforward manner. Nevertheless, there is a wealth of information about the microdialysis method. The main issue is to understand the processes that bring the chemical "signals" from the tissue surrounding the microdialysis probe, into the dialysate and the collecting vial.

In view of the discrepancy in time found between extracellular levels of DA and changes in behaviour (see below), it seems important to consider any possible delay caused by the sampling process per se. One factor that obviously determines the delay is the dead-volume of the microdialysis system, i.e., the volume from the dialysis membrane to the end of the outlet tubing. In the present experiment this dead-volume was compensated for by a delay between drug injections and changing of vials at the end of the tubing. Laminar flow in the probe and the tubing may also influence the speed by which a change in the extracellular fluid will be monitored by the system. However, theoretical calculations and experiments in vitro have shown that it takes only a few minutes to reach a new steady-state concentration in the dialysate when a sudden change takes place outside the probe {e.g., moving the probe from a beaker with a low concentration of a substance to a beaker with a high concentration and vice versa [(75), Ståhle unpublished data]}.

Another important factor is the delay caused by the diffusion process through the tissue (3,27). However, the time it takes for, e.g., DA to diffuse 0.3 mm, which is approximately the maximum distance from the probe from which DA is collected, is no more than a minute (27). Furthermore, it can be seen that the contribution from the surrounding tissue declines approximately exponentially with the distance from the dialysis membrane. For this reason, a change in the surrounding tissue will show up even quicker. Thus, from theory and experimental data it follows that any change in the dialysate probably represents changes in the tissue with no more than a few minutes delay.

There is also physiological and pharmacological evidence that changes in the dialysate occur rapidly. Induction of isoelectric EEG by means of hypoglycemia is accompanied by a peak increase in GABA levels during the first 5 min from the onset of isoelectricity (63). Inclusion of K^+ in the perfusion medium induced drastic and immediate increases in extracellular levels of DA (75), substance P (11), and several amino acids (62). These rapid changes show that the system has no appreciable delay in relation to the sampling time.

The levels of DA in the dialysate are dependent upon the concentration of Ca^{2+} in the perfusion medium (24,25), and the studies cited above show that a large number of substances are releasable by K^+. Furthermore, pharmacological manipulations that selectively affect the releasing mechanism, e.g., reserpine, have a profound effect on DA levels [(25), the present study].

Taken together, all the above support the idea that the microdialysis sampling process closely reflects changes in the extracellular fluid. There are, however, obvious limitations in our ability to extrapolate from levels in the extracellular fluid to release from nerve endings. Firstly, changes in the *release pattern* during a fraction period may not be detected because each fraction reflects only the integrated release over that period. Secondly, moderate changes in a small fraction of all nerve endings in the vicinity of the probe will not be detected because the dialysis reflects release integrated over the sampling volume. Thirdly, the measurement of release is indirect, i.e., changes in the elimination of DA from the synapse or from the surrounding extracellular fluid may affect the DA levels in the dialysate, although the release process per se is not affected. This is obvious when considering a DA reuptake blocking agent or a monoamine oxidase inhibitor which increase DA levels in the extracellular fluid without necessarily affecting release.

It may also be argued that DOPAC, a metabolite of DA, is a better indicator of DA release than DA itself (40). There is, however, substantial evidence that changes in synthesis of DA may be reflected in changes of DOPAC (75). Certain pharmacological treatments may cause a decrease in DOPAC when the release is apparently increased, e.g., amphetamine or increased K^+-levels extracellularly (75,76). We have found that αMPT (50 mg/kg) caused a larger reduction of DOPAC than APO (0.05 mg/kg), while reserpine (5 mg/kg) if anything caused a transient increase (Ståhle and Ungerstedt, in preparation). The behavioural effects of the drugs are the opposite. Thus, changes in DOPAC do not seem to be related to behaviour and it is doubtful to what extent it reflects changes in synaptic DA after, e.g., reserpine or αMPT.

Effects of APO, αMPT or Reserpine on Behaviour and Extracellular Dopamine Levels

It was expected, from the autoreceptor hypothesis, that when

the extracellular levels of DA are reduced to a given level by APO, reserpine or αMPT, the behavioural effects should be similar. The results from the present study show that αMPT (50 and 100 mg/kg; Fig. 1), reserpine (2 mg/kg; Fig. 2) and APO (0.05 mg/kg; Fig. 3a) all reduce the extracellular levels of DA to between 40% and 60% of basal levels. However, αMPT (50 and 100 mg/kg) did not suppress exploration. Reserpine (2 mg/kg) had no effect on behaviour after 3 hr, but suppressed exploration after 4 hr. APO (0.05 mg/kg) suppressed exploratory behaviour (Figs. 5, 6 and 7). Pergolide (0.005 mg/kg), which has previously been shown to suppress exploration (48), had no effect on extracellular levels of DA in the striatum (Fig. 3c). Thus, it was not possible to demonstrate the presence of a relation between reduced extracellular levels of DA and suppression of exploratory behaviour.

The fact that reserpine (2 mg/kg) did affect behaviour 4 hr after administration may be taken as evidence supporting the autoreceptor hypothesis with respect to suppression of exploration, though not for yawning because no yawning was induced by reserpine. It is, however, not easy to explain why there is a drastic change in behaviour between 3 and 4 hr after injection, while there is only a small decline in extracellular DA levels. A possible explanation is that the effect of reserpine is uneven and that, in some critical synapses, there is a large loss at 3–4 hr after injection. Another possibility is that reduced noradrenaline neurotransmission contributes to the suppressive effect on exploration by reserpine (13,59).

In the case of yawning behaviour, the discrepancy between the effects on behaviour and neurochemistry of DA agonists and αMPT and reserpine is even more obvious since neither αMPT nor reserpine can induce yawning behaviour within the first 5 hr postinjection [(36), unpublished data from this laboratory]. In addition, it has been reported that no DA antagonist induces yawning behaviour (22), and the same result has been obtained in this laboratory for SCH 23390 and raclopride (Ståhle, unpublished data). In this connection it is noteworthy that both yawning and suppression of exploration can be elicited by dopamine agonists in the presence of amphetamine (51) and that neuroleptic drugs can elicit yawning in rats treated with high doses of dopamine agonists (38) or amphetamine (51).

The dialysis experiments in the present study were performed in the striatum. We have recently found that the effects of APO on DA levels are smaller in the accumbens and the frontal cortex than in the striatum, while αMPT has approximately the same effect in the accumbens and the striatum (52). Hence, a regional variation in the sensitivity to APO is not likely to account for the present findings. This is important since it has been suggested that suppression of exploration is mediated by the nucleus accumbens (15, 16, 39, 61, 66).

Time-Response to APO, Pergolide and αMPT on Behaviour and Extracellular Dopamine Levels

The time-course for suppression of exploration (Fig. 5) and induction of yawning by APO (0.05 mg/kg) were found to have a more rapid onset and a shorter duration than the effect on extracellular DA levels (Fig. 3a,b). A similar discrepancy in the time-course for yawning and changes in DA levels was obtained with pergolide (0.02 mg/kg; Fig. 3c,d) and with EMD 23448 (50) and BHT 920 (unpublished data). The time-course for the effects of APO on exploration are consistent with findings in mice (59). Interestingly, it has been shown that the levels of APO in the rat brain follow a time-course similar to that of the behavioural effects (34,65). Thus, there is no direct relation in time between behavioural effects induced by DA agonists and the reduction of DA levels.

In a previously published article (64), we tentatively explained this discrepancy in time-course between behavioural and neurochemical effects as due to rapid changes in autoreceptor sensitivity (6, 8, 32). However, such a mechanism does not seem to explain the present findings because we have found that the yawning induced by repeated APO administration shows only slight desensitisation and suppression of exploration shows no desensitisation (30). Neither is it likely that changes in DA levels measured by means of microdialysis are delayed because of slow diffusion of DA into the probe (see above). This conclusion is supported by the present finding that the suppression of exploration following αMPT had a slower onset than the decrease of DA levels, i.e., the opposite to the findings with APO. Thus, it is concluded that the observed discrepancy in time-course between changes in behaviour and changes in the extracellular levels of DA reflects a true discrepancy in time.

Effects of αMPT, on APO-Induced Yawning and Suppression of Exploration

Pretreatment with αMPT did not shift the dose-response curve for APO-induced yawning (Fig. 4). The effect of αMPT on suppression of exploration added to that of APO, but not to pergolide (Fig. 8). It was predicted that the yawning dose-response curve would be shifted to the left and that αMPT per se would induce yawning. Thus, it seems likely that induction of yawning by DA agonists is independent of the extracellular level of DA. The same conclusion was drawn by Scheel-Krüger (41) on the basis of 6-OHDA lesions in dorsal striatum and local injections of DA agonists in the lesioned area. The reason why the effect of αMPT on suppression of exploration adds to that of APO, but not pergolide is not clear.

In conclusion, the present study shows that behavioural effects of low doses of DA agonists are not related to a reduction of the extracellular levels of DA. If the extracellular levels of DA reflect the synaptic levels of DA, this may be interpreted as evidence against the autoreceptor hypothesis. It cannot be excluded that autoreceptor mediated effects of a different nature, such as changes in release of co-transmitters (21) or changes in firing pattern (68), account for the behavioural effects of low doses of DA agonists. However, we would like to propose that these behavioural effects are mediated by a population of postsynaptic DA receptors being more sensitive to DA agonists than other postsynaptic receptor populations such as the receptors that mediate stereotyped behaviour following APO. Several explanations for the high receptor sensitivity are possible such as a large amount of spare receptors (57) or that the receptor is pharmacologically different from other DA receptors. This hypothesis has the advantage that it is reasonably simple to test.

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