stained areas exhibiting a granular aspect caused by silber deposition were observed in vascular zones (in which nerve density is the largest) and in the nuclei of brown adipocytes. Some cells identified as mast cells by their typical granulation were also strongly stained. Thus, AA does not seem to be exclusively stored in BAT innervation. Furthermore, a nuclear localization of AA in BAT is not unexpected: Chinoy and Sanjeevan[9] also found AA in nuclei of liver, adrenal and epididymis cells. As these authors consider that their technique is qualitative and cannot be used for quantitative determination, we will not discuss the localization of the AA accumulation which occurs in the BAT of cold-exposed rats.

Effects of amine administration. Neither NA, nor ISO administration induced an increase in BAT weight (table), contrary to what was previously observed[7] (in the following discussion, data obtained with rats treated by amines are compared with those obtained with animals receiving solvent). This result could be due to a smaller amount of amine being delivered by the pumps than in our previous experiments and to a shorter duration of treatment. BAT weight of rats treated with an ISO + PHE combination was significantly larger than BAT weight of control animals, but not statistically different from tissue weight of rats treated with ISO alone. Nevertheless, amine treatments affected the AA concentration of the tissue. This concentration was increased to 107% in rats receiving NA, to 121% in animals treated with ISO, and to 150% in animals receiving both ISO and PHE. The same result was obtained with GSH concentration which was significantly increased by ISO or NA administration (NA: + 36%, ISO: + 92%, ISO + PHE: + 108%). Administration of PHE alone (three separate experiments using 10^{-7} or $2 \cdot 10^{-7}$ mol · h^{-1} deliveries) did not modify AA and GSH concentrations (data not shown).

Statistical analysis of results (see table) shows that: 1) β-agonist ISO reproduced the effect of NA on AA and GSH concentrations, 2) ISO + α-agonist PHE did not induce a larger increase of AA and GSH levels than ISO alone, 3) ISO administration reproduced the effect of exposure to 5 °C on AA and GSH concentrations.

It can be concluded that the increase of NA release which occurs in the BAT of cold-exposed rats is most probably responsible for the rise of AA and GSH concentrations observed in this tissue. According to our results NA acts through a β-adrenoceptor and there is no argument for an α-adrenoreceptor role in the regulation of AA and GSH levels in BAT.

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Possible existence of a presynaptic positive feedback mechanism enhancing dopamine transmission in the anterior cingulate cortex of the rat

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Summary. A series of microiontophoretic and VTA stimulation experiments, conducted in intact, GBR-12909-treated, α-methylparatyrosine-depleted or 6-hydroxydopamine-denervated rats, provide suggestive evidence for the existence of a presynaptic, positive feedback mechanism triggered by dopamine reuptake and favoring the release of this transmitter in the anterior cingulate cortex.
Key words. Dopamine; cingulate cortex; uptake; transmission; positive feedback.

Various lines of evidence suggest an involvement of the mesocortical dopamine (DA) system in higher cognitive processes and the regulation of emotional states. a) In the rat, the ventral tegmental area (VTA) from which this system originates is one of the brain sites which most consistently supports self-stimulation[2]. b) The mesocortical DA system is metabolically activated by electric footshock stress[3]. c) Experimental lesioning of the VTA

induces a series of psychomotor disturbances including perturbations of locomotor and exploratory activities, alterations of delayed performance tasks, and disruptions of individual or social survival behaviors[4]. d) In the rhesus monkey, delayed alternation performance is impaired by regional 6-hydroxydopamine (6-OHDA) depletion of DA in the prefrontal cortex (PF)[5]. e) Several neurochemical and pharmacological studies implicate the mesocortical DA system in the etiology of schizophrenia and/or the therapeutic efficacy of antipsychotic agents[6–10].

Recent radioautographic and immunohistochemical investigations on the topographic distribution of the telencephalic DA innervation in rat, monkey and human[11–13] have emphasized the compartmentation of the mesocortical DA input into a number of subsystems innervating distinct cortical areas. In addition to arising from presumably distinct subdivisions of the VTA-substantia nigra pars compacta complex[14–17], the respective DA projections to the anteromedial or suprarhinal prefrontal (PF), anterior cingulate (ACg) and parietal (Par) regions exhibit major quantitative differences in regional and laminar distribution. Moreover, special characteristics have been assigned to the ACg as compared with the PF DA innervation, such as delayed postnatal development[18], lower endogenous DA content[19], higher DA reuptake capacity[19] and particular histofluorescence aspects of the varicosities after loading with exogenous amine[20]. In view of these observations, it seemed likely that the functional characteristics of DA transmission would also be different within the diverse territories of projection. We have explored this possibility by comparing various electrophysiological parameters of DA transmission in the PF, ACg and Par cortices of adult rats. Besides the known factors controlling the synaptic efficacy of monoamine transmitters in the CNS (firing rate, transmitter utilization and turnover, reuptake, pre- and postsynaptic receptors), the results suggest the existence in ACg cortex, but not in PF or Par, of a presynaptic positive feedback mechanism triggered by DA release and enhancing the further liberation of this amine.

Materials and methods

Microiontophoretic experiments were carried out in adult male Sprague-Dawley rats (225–250 g b. wt) anesthetized with urethane (1.5 g/kg i.p.). Three groups of rats were examined: 1) intact rats (n = 15) tested before and 5 to 90 min after a single dose of the DA uptake inhibitor GBR 12909 (GBR, 500 µg/kg i.v.); 2) rats pretreated with the inhibitor of DA synthesis alpha-methyl-paratyrosine (α-MPT; n = 6) (200 mg/kg i.p., 18 and 2 h before), and 3) rats subjected to unilateral DA denervation of the cortex with 6-OHDA 2–4 weeks earlier (n = 4). Two microinjections of 6-OHDA (8 µg p.w. each) were made one hour after desipramine pretreatment (25 mg/kg i.p.) at stereotaxic sites A 3.8, L 0.8 and H + 2.0 mm for the VTA, and A 3.8, L 2.4 and H + 1.8 mm for the substantia nigra pars compacta[21], according to Doucet at al.[22]. DA depletions of more than 95% and 70% were measured by HPLC in both ACg and PF, after α-MPT treatment and 6-OHDA denervation, respectively.

For microiontophoresis, five barrel micropipettes were used as previously described[23]. The ejection barrels were filled with either 0.5 M DA, the D1 agonist SKF 38393 (0.01 M) or the D2 agonist 2-(N-phenylethyl-N-propyl)-amino-5-hydroxytetralin (PPHT; 0.005 M), adjusted at pH 4. All three agents predominantly induced inhibitions of spontaneous neuronal firing (depressions ≥ 50%). Neuronal presponsiveness was quantitatively assessed in the deep layers of ACg, PF, Par, and in the neostriatum (NS), by means of a recuperation time index ($R{\cdot}T^{90}$). In each region and condition tested, the ejecting current was kept at the level found to induce a 50% inhibition of firing, and the time interval was determined between the offset of iontophoretic ejection and the return to 90% of the initial discharge rate.

To evaluate responsiveness to endogenously released DA, we also examined the effects of an electrical stimulation of the VTA on the spontaneous firing rate of ACg and PF neurons in urethane-anesthetized rats[24]. These recordings were also obtained from the deep layers, using glass microelectrodes. A concentric bipolar electrode was implanted in the VTA and 0.5-ms pulses were delivered during 150 s at a current intensity of 600 µA and a frequency of 1 Hz. Peristimulus time histograms were constructed in both control and GBR-treated rats. Here again, inhibition was defined as a depression of at least 50% of the spontaneous firing rate. The duration of inhibition was measured as the time interval after stimulation during which none of the bins showed more than 50% of the average number of spikes in the control, prestimulus period (200 ms). The decrease in firing was calculated by subtracting the number of spikes in the inhibition period from the average in the same length of time during the control period, and expressed as percent of control.

Results

In intact, α-MPT-depleted and 6-OHDA-denervated cortex, the amount of DA required to obtain a 50% reduction in the spontaneous firing rate was nearly identical for all regions studied, precluding major differences in the postsynaptic sensitivity to this amine. Since neuronal uptake is the major mechanism of inactivation of DA in CNS, we were anticipating in the intact cortex shorter effects of microiontophoresed DA in the relatively densely DA-innervated ACg and PF regions, as opposed to the sparsely innervated Par region. However, the average duration of DA inhibitions was 5-fold higher in ACg than in either PF or Par, and similar in the latter two regions (fig. 1). After intravenous administration of

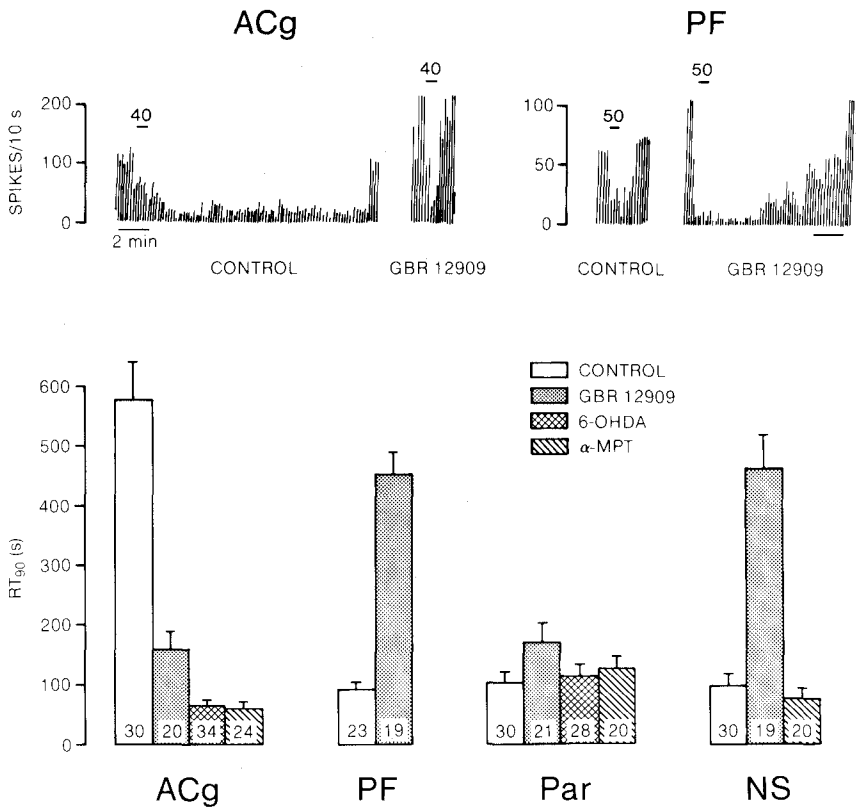

Figure 1. Effects of DA reuptake blockade with GBR 12909, inhibition of DA synthesis with α-MPT, and 6-OHDA denervation, on the responsiveness to microiontophoresed DA in anterior cingulate (ACg), medial prefrontal (PF) and parietal (Par) cortices and in the neostriatum (NS). The upper traces exemplify the typical effect of GBR 12909 administration in the intact ACg and PF cortices. As illustrated here, different units had to be recorded before and after the treatment. Bars with numbers above indicate the duration of DA application with the ejection current given in nA. The frequency of discharge is integrated over 10-s intervals. Note the longer duration of DA inhibition in ACg compared to PF and the opposite effects of GBR 12909 in these two cortical regions. The histograms below depict the average duration of DA inhibitions recorded from the three cortical regions and the NS under the different conditions examined. Recovery time from the offset of inhibition to 90% of the initial spontaneous firing frequency (RT90) was measured from the number of units indicated at the bottom of each column. A Student's t-test revealed significant differences between control responses in ACg and the three other regions ($p < 0.01$), between control and responses after all three treatments in ACg ($p < 0.01$), and before and after GBR 12909 administration in PF and NS ($p < 0.01$).
The stereotaxic coordinates[21] for these recordings were as follows. ACg: A 9.5–11, L 0.5–1.0 and H + 7.0–8.5 mm; PF: A 12–13, L 0.3–0.4 and H + 6.5–7.5 mm; Par: A 6–8, L 2–4 and H + 8.0–9.5 mm; NS: A 9.0–10.5, L 1.5–3.5 and H + 3.5–6.5 mm.

GBR 12909, a very potent and highly selective blocker of DA uptake[25], the duration of the DA inhibitions appeared to be significantly enhanced in PF but, surprisingly, was reduced 4-fold in ACg (fig. 1), as if the reuptake process was in some way responsible for the prolonged effects of DA in ACg. Accordingly, GBR appeared to be without significant effect in the sparsely DA-innervated Par cortex, and yet had an effect similar to that observed in PF in the extremely densely DA-innervated NS (fig. 1). In none of the regions studied did GBR affect the average spontaneous firing rate. To test for a direct effect of GBR on postsynaptic DA receptors in ACg, responsiveness to the iontophoretic application of the DA agonists SKF 38393 (D1) and PPHT (D2) was assessed before and after the administration of this uptake blocker. No difference was then noticed in the dose required to induce 50% inhibitions and the duration of these responses with either agonist, which precluded an interaction of GBR with DA postsynaptic receptors.

Since GBR could have acted through presynaptic mechanisms other than DA reuptake in ACg, we proceeded with the examination of DA responses after selective deafferentation of the mesocortical DA system by 6-hydroxydopamine. Unilateral lesioning of the VTA and SN caused a 9-fold decrease in the duration of the DA inhibition in ACg, but remained without effect in Par (fig. 1). In view of the apparent lack of postsynaptic effect of GBR in intact cortex, this paradoxical effect of the DA denervation in ACg seemed also imputable to the suppression of the DA reuptake mechanism. Altogether, these results raised the possibility that, in ACg, DA transported back into DA terminals could provoke a further liberation of either DA or of another chemical responsible for the prolongation of DA effects.
To tackle this issue, the series of experiments after α-MPT depletion was carried out. As with 6-OHDA denervation, this biochemical reduction of endogenous DA resulted in 9-fold reduction of the duration of inhibitory responses

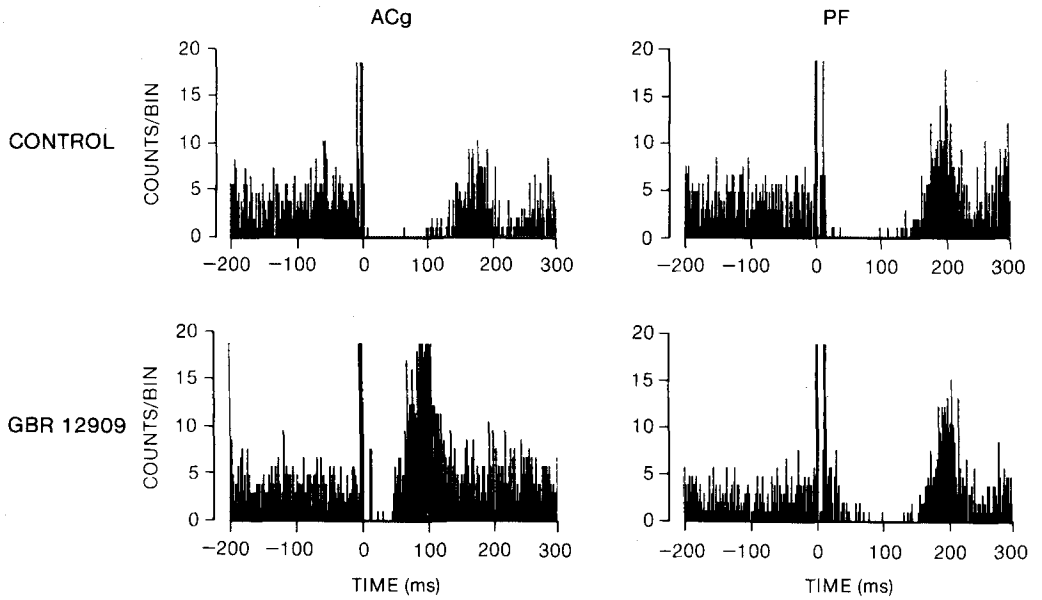

Figure 2. Effect of GBR 12909 on the inhibition induced by VTA stimulation in the ACg and the PF cortices. Peristimulus time histograms were constructed from recordings of spontaneously firing units (number of cells in the table). Each histogram represented 150 stimuli. Bin width was 2 ms and the total observation period was 500 ms. The stimulation pulse was delivered at time 0. The pulses generated by the stimulator and a differential amplitude discriminator triggered by the unitary activity of the recorded neurons were digitized and stored on magnetic media. The peristimulus time histograms were subsequently analyzed with a 6502 microprocessor (Apple IIE). An electrolytic lesion (0.5 mA for 7 s) was produced at the end of each experiment to verify histologically the location of the stimulating electrode.

Effect of VTA stimulation on the spontaneous activity of ACg and PF neurons in control and GBR 12909-treated rats

	ACg		PF	
	Control	GBR 12909	Control	GBR 12909
Number of cells tested	44	50	48	50
Responsiveness (%)	77	56 †	62	64
Decrease in firing (%)	81 ± 4	80 ± 3	84 ± 3	85 ± 3
Latency of response (ms)	23 ± 2	36 ± 5*	31 ± 2	36 ± 6
Duration of inhibition (ms)	72 ± 5	57 ± 4*	82 ± 6	84 ± 5

† Chi-square test: $p < 0.05$; *Student's t-test: $p < 0.05$.

to microiontophoresed DA in ACg, but no significant change in either Par or NS (fig. 1), strongly implicating DA itself in its prolonged effect in normal ACg.

The question was also raised whether endogenously released DA would have a similar action as microiontophoretically applied DA in ACg cortex. As assessed by the responsiveness of cortical neurons to VTA stimulation before and after GBR administration, endogenous and exogenous DA seemed to produce comparable effects. Following GBR treatment, the number of cells inhibited by VTA stimulation was reduced in ACg, but not in PF, and the remaining responses had an increased latency and shorter duration (fig. 2 and table).

Discussion

The foregoing results led us to postulate the existence of a presynaptic positive feedback mechanism which is triggered by DA and dependent on the DA reuptake process, and which might stimulate the further liberation of this transmitter upon its initial release by ACg axon terminals. A direct postsynaptic mechanism was excluded since, at least in ACg, neither GBR administration, α-MTP depletion or 6-OHDA denervation modified the initial sensitivity to DA as evaluated with the IT50 index (iontophoretic current x time required to obtain a 50% depression of firing). Nor was the responsiveness to DA agonists affected by GBR, either in terms of initial sensitivity (IT50) or of the duration of these inhibitory responses (RT90), which would have been expected from a change in DA postsynaptic receptors or effector mechanisms. In considering possible presynaptic mechanisms, the fact that the amount of exogenous DA necessary to induce 50% inhibitions was the same in the intact cortex and after DA depletion or denervation ruled out a significant implication of endogenous DA in this initial component of the response.

In contrast, the shorter duration of inhibitions obtained in ACg after both α-MPT and 6-OHDA as well as GBR administration suggested that DA availability could be a major factor determining the length of the responses. In the absence of a similar effect of these treatments on the enzymatic degradation or reuptake of DA, and since the quantity of microiontophoresed DA was kept relatively constant in all experiments, a further release of endogenous DA following the initial action of this amine had to be postulated to account for the prolonged duration of DA inhibitions in the intact ACg. The fact that GBR could paradoxically decrease the duration of these re-

sponses in the intact cortex led us to envisage that DA reuptake might be the triggering event in the postulated feedback mechanism. An alternative explanation could have been that all treatments reduced a tonic release of DA associated with the high turnover of this transmitter in the intact ACg cortex. However, this eventuality was highly unlikely in view of current evidence indicating that 1) mesoprefrontal DA cells lack somato-dendritic impulse-modulating autoreceptors[26,27]; 2) GBR is apparently devoid of DA releasing effects at least in the neostriatum[28]; 3) in neostriatum, another DA reuptake blocker, benztropine, increases the in vivo release of DA as measured by the push-pull perfusion technique[28]; 4) the specific DA autoreceptor agonist, EMD 23448, enhances the basal release of DA from slices of the prefrontal cortex preincubated with [^{3}H]DA[29].

As already noted by Wolf and Roth[30], some DA agonists have previously been reported to enhance the basal efflux of radioactivity from slices of the striatum preincubated with [^{3}H]DA, apparently as a result of their ability to enter the nerve terminal and storage vesicles an subsequently accelerate the efflux of DA and DA metabolites[31]. It remains to be determined whether an intracellular mechanism of this type may be triggered by DA to account for a further release of DA provoked by DA reuptake into ACg terminals. Owing to the low endogenous DA content of mesocortical DA terminals, the DA pools in ACg cortex are probably small enough to be sensitive to changes in release. It is therefore reasonable to assume that the homeostasis of the mechanism is ensured through release-dependent alterations in intraneuronal DA levels.

By favoring the release of DA in ACg cortex, the postulated positive feedback mechanism might explain, at least in part, the higher rate of DA utilization and turnover measured in this cortical region when compared to NS[19,32]. Because GBR produced similar reductions of responses induced by electrical stimulation of the VTA, and thus by endogenously released DA, the postulated positive feedback could be operative in the intact cortex. The fact that an equivalent activation of different sets of mesocortical DA neurons might have greater effects in ACg than other regions of the cortex could have significant implications for our current understanding of DA function in this part of the brain. Since recent studies have demonstrated that the mesocortical DA system undergoes a major development during phylogeny[13], it would be of interest to determine which component(s) of the DA cortical innervation is(are) subject to positive feedback regulation in higher species. In view of the interconnections between DA-innervated cortical regions[11], the above described positive feedback mechanism could intervene in many of the functions or dysfunctions currently ascribed to the mesocortical DA system. In considering the possible use of selective DA uptake blockers as therapeutic agents, it would be important to envisage the possibility that these might exert effects in ACg opposite to those in other cortical and subcortical regions.

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