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Research report

Dopamine release in the rat globus pallidus characterised by in vivo microdialysis

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Abstract

Brain microdialysis has been used to examine the in vivo effects of potassium and calcium on dopamine release in the dorsal globus pallidus (GP) of rats. Furthermore, the effects of food presentation and consumption on dopamine release in the GP were investigated. Basal dopamine levels in the GP were below the detection limit, therefore nomifensine (30 μM) was added to the perfused artificial cerebrospinal fluid (aCSF). A prominent increase of dopamine release to 370% was observed after perfusion with elevated potassium (100 mM), while perfusion with calcium-free aCSF produced a significant decrease of dopamine efflux to 36% of control levels. Furthermore, presentation and consumption of food resulted in a rapid increase of extracellular dopamine to 130%. The present experiments demonstrate that in the GP extracellular dopamine can be measured by in vivo brain microdialysis. The data suggest that the dopamine release in the GP can be stimulated by a depolarising agent and involves a partially calcium-dependent release mechanism. The data further suggest that dopamine in basal ganglia structures downstream the striatum as the GP is involved in signalling of important stimuli in the environment, e.g. food.

Keywords: Globus pallidus; Dopamine; Food; In vivo brain microdialysis

1. Introduction

In the current models of the functional organisation of the basal ganglia, the GP is considered as an indirect relay linking the striatum to the output structures of the basal ganglia, i.e. the nucleus entopeduncularis and the substantia nigra pars reticulata in the rat. The projections from the GP to these output structures as well as to the subthalamic nucleus and to the reticular thalamic nucleus use γ-amino butyric acid (GABA) as transmitter [20]. Pallidal afferents from the striatum are GABAergic, while afferent projections from the subthalamic nucleus and the parafascicular nucleus are glutamatergic [8,20]. In addition, the GP receives a small dopamine projection by collaterals of nigrostriatal fibers [16]. There is growing evidence that the GP plays a key role in motor control of the basal ganglia [1,11].

An important aspect of understanding pallidal motor functions which has been largely neglected is the significance of its dopaminergic innervation. A major role of dopamine in control of pallidal motor circuits is suggested by findings that an intrapallidal dopamine receptor blockade produced massive akinesia in rats [2,13] and increased neuronal activity in the output structures of the basal ganglia as shown by immunohistochemistry [12]. Furthermore, neurochemical cell body lesions in the GP reduced dopamine receptor-dependent motor behaviour, probably by destroying dopamine receptor bearing neurons [14]. At present there exist in vivo measurements of the dopamine release only in the ventral pallidum (VP) [10], but not in the GP. Therefore, the objective of the present study was to analyse basic pharmacological and physiological characteristics of pallidal dopamine release by brain microdialysis in awake, freely moving rats.

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2. Materials and methods

2.1. Subjects

Protocols for animal experiments described in this study were performed according to the national laws on animal experiments and were approved by the proper authorities.

Male Sprague–Dawley rats (Charles River, Germany) weighing 200–250 g on arrival were housed in groups of 4–5 animals in transparent plastic cages (type IV) in a colony room (temperature: 21 ± 2°C; relative humidity: 55 ± 5%). Animals were maintained on a 12:12 h light–dark cycle with lights on at 06:00 h. Food (rodent maintenance diet, Altromin, Germany) was restricted to 15 g/day and per animal, water was freely available.

2.2. Stereotaxic surgery

Intracranial, silikonized guide cannulae (CMA/12; outer diameter: 0.9 mm) (CMA, Sweden) were implanted unilaterally and aimed to the GP. The co-ordinates relative to bregma were: AP -1.3 mm; L 3.1 mm in either the right or left GP; V -5.2 mm from dura with the incisor bar set 3.3 mm below the interaural line [23]. Surgery was performed with a stereotaxic frame (David Kopf Instruments, USA) using standard stereotaxic procedures under sodium pentobarbital anaesthesia (60 mg/kg i.p., Sigma, Germany) with atropine sulfate pretreatment (0.5 mg/kg i.p., Sigma, Germany). After surgery animals were housed individually in Macrolon® ($37 \times 21 \times 30$ cm) cages (type III) with raised solid-walled lids.

2.3. Microdialysis

Microdialysis experiments started at least one week after surgery and were performed in a plexiglas bowl (CMA, Sweden). A microdialysis probe (CMA/12, Sweden; 2 mm exposed membrane length, 0.5 mm outer membrane diameter) was inserted through the guide cannula and connected via a swivel (Instech, USA) with a CMA/100 microdialysis pump (CMA, Sweden). Probes were perfused with aCSF (147 mM Na^+, 2.5 mM K^+, 2.2 mM Ca^{2+}, 0.9 mM Mg^{2+}, 155.7 mM Cl^-) and 30 μM nomifensine [Research Biochemicals Inc., USA]). For potassium stimulation, aCSF was used with 100 mM K^+ and 47 mM Na^+ (rest unchanged). Calcium-free aCSF was produced by omitting $CaCl_2$. Nomifensine was added from aliquots of a frozen (-75°C) stock solution in aCSF. For calcium-free aCSF, bidestilled water was used for the stock solution. Perfusion flow rate was 2.5 μl/min and dialysate samples were collected every 30 min.

2.4. High pressure liquid chromatography (HPLC)

Dialysates were analysed for dopamine using reversed-phase ion-pair HPLC with electrochemical detection. The mobile phase (pH 3.7) consisted of 60 mM NaH_2PO_4, 0.4–0.5 mM octanesulfonic acid, 0.14 mM EDTA and 15% (v/v) methanol. Sample run time was < 22 min at a flow of 0.35 or 0.4 ml/min. A Nucleosil 100-5-C18 column (5 μm particles, length × i.d. 125×3 mm, Bischoff, Germany) was used combined with a precolumn (length 20 mm). Electrochemical detection was performed with a VT-03 electrochemical flow cell and an INTRO detector (Antec, Netherlands). The working electrode was set at $+800$ mV (experiment 1) and $+600$ mV (experiment 2) against an Ag/AgCl reference electrode. The HPLC apparatus consisted of a Kontron 520 pump and a Kontron 465 autosampler (Bio-Tek Kontron, Germany). Data were recorded by a MT2 Data System (Bio-Tek Kontron, Germany). The detection limit (signal to noise > 3) for dopamine was about 3 pg/injection.

2.5. Experimental procedures

In the first experiment ($n = 6$), we studied GP dopamine release and the effects of perfusion with elevated potassium and calcium-free aCSF. The experiment was carried out between 12:00–02:00 h during constant light. Four hours after probe insertion, aCSF was changed to elevated potassium aCSF for 30 min and another 4 h later the probe was perfused for 2 h with calcium-free aCSF. In the second experiment ($n = 5$), we tested the effects of a physiological stimulus, i.e. food. The experiment was performed during 08:30 and 20:30 h under constant light. Seven and a half hours after probe insertion and continuous perfusion with aCSF, standard food pellets (rodent maintenance diet, Altromin, Germany) were given to 24-h food deprived animals for 30 min.

2.6. Histology

After the experiments, animals were sacrificed by an overdose of sodium pentobarbital and the brains were fixed for 2 h in 10% (v/v) formalin and immersed in 30% (w/v) sucrose. 40 μm sections were taken by a cryostat (Leica, Germany) and stained with cresyl violet to verify probe location.

2.7. Statistics

Data are expressed and statistically analysed as follows [29]: all values are expressed as percentages of controls. The average concentrations of three samples (deviation < 10%) immediately before perfusion with elevated potassium aCSF and before presentation of

food were considered as respective control values and were defined as 100%. Data were analysed (SigmaStat Vers. 2.0, SPSS Inc., USA) by a nonparametric one-way ANOVA for repeated measurements followed by Dunnett's multiple comparisons test. The level of significance was set at $P < 0.05$.

3. Results

Extracellular dopamine levels in the GP were at the limit of detection (< 3 pg/sample) precluding reliable measurement of the basal release. Therefore, the catecholamine reuptake blocker nomifensine (30 μM) was added to the perfusate [7,18,24]. Thereafter, basal values of dopamine were reliably detected. For instance, the mean dopamine level (± SEM) of the last control sample before food presentation was 40.9 ± 4.3 pg/sample ($n = 5$).

Results of the first experiment demonstrate that perfusion of potassium (100 mM) for 30 min led to a significant, about 3.7-fold increase in dialysate dopamine content ($P < 0.05$, $n = 6$) (Fig. 1A). Furthermore, perfusion with calcium-free aCSF revealed that dopamine efflux was at least partially calcium-dependent (Fig. 1A): Compared to the control value ($T = 270$ min) immediately preceding the first calcium-free sample, dopamine efflux was significantly reduced during perfusion with calcium-free aCSF for 120 min (maximally to 36% of the control value) ($P < 0.05$, $n = 6$) and increased immediately after reperfusion with normal aCSF. A moderate increase of dopamine baseline levels was observed over the entire microdialysis period of 10 h and dopamine dialysate contents of the last three samples were significantly higher than the control value ($T = 0$ min) ($P < 0.05$, respectively). In the second experiment, we tested the effect of food presentation and consumption on pallidal dopamine release in 24 h-food deprived rats. After presentation of food at the beginning of the sample interval, animals ($n = 5$) which were inactive or sleeping before, became active and started to eat within 5–10 min until food was removed at the end of the sampling period. Extracellular dopamine increased significantly during food consumption (Fig. 1B) and subsided about 60 min later. As in experiment 1, there was a moderate increase in the dopamine baseline levels over the entire microdialysis period. Fig. 2 shows the location of microdialysis probes of all animals used in both experiments. Only rats with proper probe location were included in Fig. 2 and in the data evaluation.

4. Discussion

Using in vivo microdialysis in awake, freely moving rats, the present experiments demonstrate to our knowledge for the first time a partially calcium-dependent dopamine release in the GP which can be increased by perfusion with elevated potassium aCSF or by an incentive stimulus, i.e. food.

Basal dopamine levels in the GP monitored by microdialysis are relatively low and can not be reliably measured with an HPLC system with a detection limit of about 3 pg/injection as already reported earlier [4]. Therefore, we used the catecholamine reuptake blocker nomifensine to increase dopamine levels as described in numerous studies (e.g. [7,18,25]). The dose of nomifensine we used has been shown to produce a robust increase in extracellular dopamine [18]. There is consistent evidence that nomifensine does not alter major pharmacological characteristics of the dopamine release, i.e. its calcium- and tetrodotoxin-sensitivity [3]. Furthermore, the dopamine release induced by physiological stimuli is comparable whether or not nomifensine is added to the aCSF (see [7]): For instance, increases of extracellular dopamine levels induced by stress in the prefrontal cortex, or by food consumption in the nucleus accumbens are similar in the presence or absence of nomifensine.

Experiment 1 demonstrates that perfusion of the GP with elevated potassium aCSF induced a significant increase of dopamine efflux. This finding is in keeping with the demonstration of a potassium-evoked dopamine release in a slice preparation of the rat GP [27]. The magnitude of the potassium-induced dopamine increase in the GP is comparable to the striatal or nigral dopamine release induced by similar concentrations of potassium [6,25]. Thus, dopamine release in the GP can be elevated by a depolarising stimulus like potassium which is likely to be mediated by a carrier-independent, presumably vesicular process [6]. Furthermore, removal of calcium from the aCSF produced a decrease of dialysate dopamine content to 36% of controls which corresponds well with similar findings obtained in the striatum [5,19]. Therefore, it is concluded that the extracellular dopamine measured in our preparation is largely derived from a calcium-dependent vesicular process. There was a moderate increase of baseline dopamine levels in our preparation over the entire experiment. One explanation could be that because of the continuous local reuptake blockade there is an accumulation of extracellular dopamine in the GP. Alternatively, due to the long microdialysis period, this might reflect increases of extracellular dopamine in the GP during the light-dark cycle like those described in the caudate-putamen [21,22]. This question will be addressed in further studies using an HPLC method with an improved sensitivity in order to perfuse aCSF with reduced nomifensine concentrations or even without nomifensine. Finally, we studied the effect of a physiological stimulus known to activate the ascending dopamine systems, i.e. food. The relative increase in

extracellular dopamine (130–200% of controls) induced by food has been shown to be particularly prominent in the nucleus accumbens and prefrontal cortex, but also in the caudate-putamen [7,28]. Likewise, we observed a food-induced increase of pallidal dopamine efflux to about 130% of controls. It is unlikely that the experimental conditions in our study interfere with the food-induced dopamine release in the GP. The relative increases induced by feeding are known to be similar, irrespective whether rats were perfused using aCSF with or without a dopamine reuptake blocker [28]. Furthermore, dopamine release during food intake experiments seems to be related to the initiation of eating and not to food-related motor activity [28]. Thus one may conclude that an incentive stimulus like food does not only increase dopamine activity in the nucleus

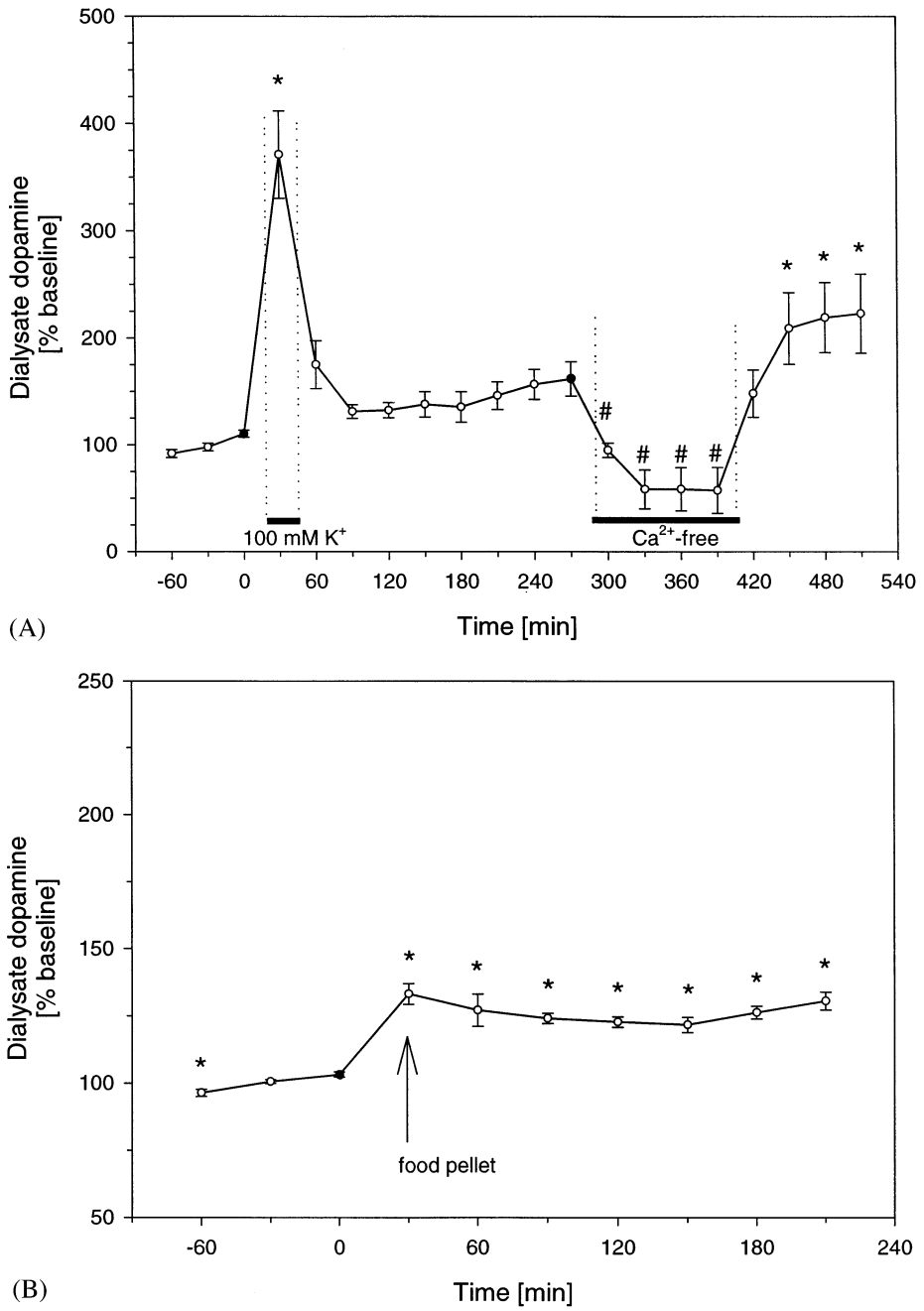

Fig. 1. (A) The effect of perfusing with elevated potassium (100 mM, 30 min) and calcium-free (120 min) aCSF containing nomifensine (30 μM) on dopamine efflux in the globus pallidus (expressed as percent of basal values ± SEM). * $P < 0.05$ versus $T = 0$ min (filled circle); # $P < 0.05$ versus $T = 270$ min (filled circle). $n = 6$. (B) The effect of presentation and consumption of food pellets (30 min) in 24-h food-deprived rats on dopamine efflux in the globus pallidus (expressed as percent of basal values ± SEM). ACSF contained nomifensine (30 μM). * $P < 0.05$ versus $T = 0$ min (filled circle). $n = 5$.

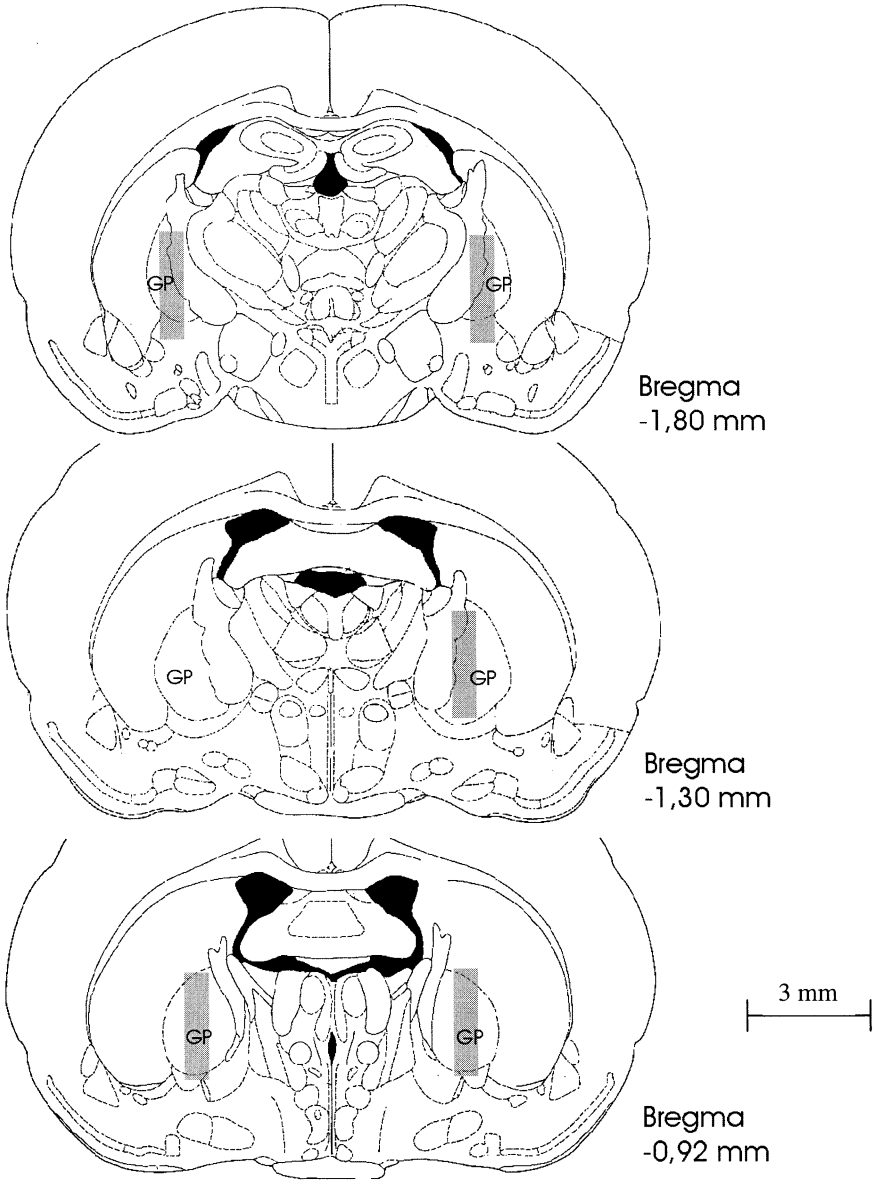

Fig. 2. Schematic diagram showing the histologically confirmed location of the probes reconstructed on the basis of serial coronal sections derived from the atlas of Paxinos and Watson [23]. The positions of the exposed probes of all animals from both experiments were within the shaded areas. GP, globus pallidus.

accumbens, caudate-putamen or prefrontal cortex [7,28] but also in the GP. Therefore, the present data provide evidence that the dopamine innervation of the GP might share two major characteristics of ascending dopamine systems: first, a tonic mode of operation which might serve to enable a wide range of behaviours; second, a phasic mode of operation which might be related to signalling of environmental motivating stimuli [26]. Behavioural studies provide evidence in favour of an enabling function of dopamine in the GP, because a stimulation of pallidal dopamine receptors increased locomotor activity [17] and produced stereotyped movements [9], while a blockade induced akinesia in rats [2,13]. Furthermore, massive reductions of dopamine levels in the lateral GP (the primate homologue to the rat GP) in parkinsonian patients most likely contribute to hypokinesia in this disease [15].

In summary, the present study shows by in vivo brain microdialysis that GP dopamine release can be stimulated by a depolarising agent and involves a partially calcium-dependent release mechanism. Furthermore, GP dopamine seems to contribute to signalling of important stimuli in the environment which subserves adaptive control of behaviour [26] and might, as shown here, involve basal ganglia structures downstream the striatum.

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