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Two simultaneously working storage pools of dopamine in mouse caudate and nucleus accumbens

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1 The dynamics of the decline of evoked dopamine overflow after repeated electrical stimulation (2 or 4 s train duration, 50 Hz) of the median forebrain bundle were investigated by means of *in vivo* voltammetry in mouse caudate and nucleus accumbens. An unexpected effect – slowing of the rate of dopamine decline after repeated stimulation at short (10 s or less) between-stimulation intervals, and an increase in the absolute amount of dopamine released at the beginning of the repeated stimulation – was found.

2 After the evoked dopamine overflow had been reduced by α-methyl-p-tyrosine (AMPT), repeated stimulation at intervals of 5 s increased dopamine release to each subsequent stimulation applied.

3 It is proposed that there are two compartments involved in dopamine storage. Both contribute to the release of dopamine, however, they may be separated artificially by either applying stimulation at short intervals or by depletion of intracellular dopamine.

4 The first releasable pool (newly synthesized, AMPT-sensitive) provides dopamine for the release after a single stimulation or repeated stimulation, being independent of whether the first stimulation is succeeded quickly by a second. It is also independent of between-stimulation interval.

5 The second pool (AMPT-insensitive storage pool) is progressively activated after repeated stimulation. The duration of the between-stimulation intervals is the crucial factor for the activation of this pool.

Keywords: Dopamine; storage pools; nucleus accumbens; caudate nucleus; *in vivo* voltammetry

Introduction

Since stability of evoked dopamine release in the terminal fields after repeated electrical stimulation of ascending dopaminergic pathways is essential for pharmacological studies, most investigations of this topic have examined the conditions which maintain stable dopamine release after repeated stimulation. Indeed, a gradual decrease of the peak dopamine overflow with decrease of time between serial stimulations would be predicted because of non-compensated depletion of dopamine storage. Generally, the less time between stimulations that has been used, the more profound the decrease in the evoked dopamine release to each following stimulation (Michael *et al.*, 1987a,b). Inhibition of dopamine synthesis by α-methyl-p-tyrosine (AMPT) increases the time needed for recovery of the evoked dopamine overflow. However, this relationship was found only for intervals from 2 to tens of minutes. At shorter (30 s to 2 min) time intervals, it was noted that the dynamics of dopamine recovery are not sensitive to inhibition of dopamine synthesis by AMPT (Michael *et al.*, 1987a). Moreover, amfonelic acid, which effectively restores stimulated release inhibited by AMPT (Ewing *et al.*, 1983), had no action on the dynamics of repeated dopamine release with only a 2 min interval between stimulations (Michael *et al.*, 1987b). Thus, the regulation of the dopamine recovery at short between-stimulation intervals may involve some additional mechanisms.

It has been proposed that there are at least two compartments for dopamine storage in the striatum (Javoy & Glowinski, 1971; McMillen *et al.*, 1980; Ewing *et al.*, 1983; Schoemaker & Nickolson, 1983). One compartment is a functional (releasable) pool, which provides dopamine for normal neuronal activity. The second pool is a non-functional intraneuronal storage pool or a reserve compartment. Unfortunately, there is little data relating to *in vivo* activation of this reserve compartment in the striatum. Ewing *et al.* (1983), using *in vivo* voltammetry, have shown that amfonelic acid administered subsequently to inhibition of electrically stimulated release of dopamine by AMPT, significantly (75% of its initial value) increased the amount of evoked dopamine release in caudate nucleus. They suggested that in the 'emergency' situation of electrical stimulation, the reserve compartment might be mobilized and that amfonelic acid either facilitated transfer between compartments or facilitated direct exocytosis of dopamine stored in the nonfunctional compartment.

Up till now, the dynamics of dopamine recovery have only been investigated for intervals of 30 s or more between stimuli. However, recent improvements in the time resolution of *in vivo* voltammetry permit investigations into dopamine release and recovery at even shorter between-stimulation intervals.

When the parameters of repeated electrical stimulation of median forebrain bundle on the dynamics of dopamine release in mouse striatum were studied, an unexpected effect was observed. When the time between stimulations was reduced to 10 s or less, a relative increase in dopamine overflow to each following stimulation occurred. It is proposed that this effect may be related to the function of dopamine storage pools.

Methods

Preparation of animals

All experiments were carried out on male BALB/c mice (25–30 g), bred in the National Animal Centre, Kuopio, Finland. Mice were anaesthetized with chloral hydrate (450 mg kg^{-1}, i.p.) and fixed in a stereotaxic frame. Anaesthesia was maintained at a level sufficient to prevent corneal reflexes by repeated injections of the drug at 100 mg kg^{-1} every 30–40 min. Rectal temperature was kept at 37°C with a heating blanket. After the removal of a small region of the skull with a dental drill and opening the dura, a voltammetric detector (working electrode) was placed in the caudate nucleus (AP:

0.5 mm, L:2.0 mm, V: −3 mm vs. bregma and cortical surface) or nucleus accumbens (AP: 1.4 mm, L: 1.0 mm, V: −4.0 mm) according to stereotaxic atlas of the mouse brain (Slotnick & Leonard, 1975). A miniature silver/silver chloride reference electrode in a saline bridge was positioned on the same side of the skull at about 2 mm from the working electrode in connection to saline solution which always bathed the open skull. The auxiliary electrode (stainless steel screw) was embedded in nose bones. All procedures used were approved by the local animal care committee.

Electrical stimulation

A laboratory built concentric bipolar stimulating electrode with active tip 0.2 mm thick and 0.3 mm long was implanted in the median forebrain bundle (MFB) (AP: −2.0 mm, L: 1.2 mm, V: −4.7–4.9 mm vs. bregma and cortical surface). The electrode was lowered 0.3 mm above the region to be stimulated and stimulation started while the electrode was slowly advanced (1 mm min^{-1}). The stimulating electrode was fixed at a depth where the oxidation current at the working electrode was maximal. At least two additional stimulations at 10 min intervals were given to ensure that baseline and peak oxidation current were stabilised. Electrical stimulation was a constant-current sine wave at 50 Hz, electronically switched on for a 2 or 4 s period and synchronised with voltammetric measurements to avoid artefacts. The root mean square current was normally kept at 80 μA. Two types of experiments and stimulations were performed. In the first the time-courses of the effects of drugs upon the evoked overflow of dopamine were investigated. Electrical stimulations (4 s train duration) were applied to the MFB at intervals of 10 min throughout the experiment. Drugs were administered immediately after the first stimulation and their effects monitored for about 2 h. In the second type of experiment, the dynamics of evoked dopamine overflow and the effects of drugs after repeated electrical stimulation at short intervals (30, 10 and 5 s) were investigated. One train of electrical stimulation (2 or 4 s train duration) was applied to the MFB before administration of drugs or saline. One hour after administration, repeated electrical stimulation was applied to the MFB. A 4 s train duration was used for the 8 repeated stimulations at 10 and 30 s intervals. Since stimulation for 4 s induced a peak increase of oxidation current which lasted about 9–10 s, the train duration was shortened to 2 s for stimulation at 5 s intervals. The stimulations at 5 s intervals were organized into 5 'bursts' (6 stimulations in the burst) with 60 s between-burst intervals.

Electrochemical technique

A single carbon fibre (Courtaulds Grafil XA-S, 8 μM in diameter) embedded in borosilicate glass as described by Armstrong-James and Millar (1979) was used as a working electrode. The end of the fibre protruding beyond the glass insulation was trimmed to a length of 300–350 μm. The crevices between the carbon fibre and glass insulation at the tip were sealed with epoxy resin. The custom-built three-electrode potentiostat system was connected to an IBM computer with digital-analogue and analogue-digital converters for the recoding and storage of data and generation of input waveform used with this carbon fibre electrode. The catechol oxidation current was monitored by high-speed chronoamperometry at +0.55 V vs. Ag/AgCl electrode every 0.25 s. The computer generated input waveform was a 0.0–0.55 V square pulse, 50 ms width, with current integration from 25 to 48 ms. For the registration of the reduction current, a similar 50 ms pulse at +0.55 V with 50 ms back slope to 0.0 V and current integration from 75 to 98 ms was used. Following the experiments, the electrodes were calibrated in solutions of dopamine (1 and 10 μM) and ascorbic acid (200 μM) in phosphate buffered saline.

Data presentation and statistics

The amplitude of the dopamine overflow was measured as the height of perpendicular drawn from the line between the shoulders of the peak oxidation current to the apex of the peak. Data from the three types of experiments are presented. Time courses of the drug effects upon the evoked dopamine overflow (Figure 1b–d) are shown as averaged data (mean ± s.e.mean), expressed as a percentage of the first stable evoked dopamine overflow following the stimulation of the MFB. Drugs were injected immediately after this stimulation. The dynamics of the decline in dopamine release after repeated stimulation at short intervals are shown as averaged data (mean ± s.e.mean) expressed as a % of the amount of dopamine following the first stimulation-evoked stable overflow. The effects of drugs on the dynamics of dopamine decline after repeated stimulation are shown as a % of the evoked dopamine overflow after the first stimulation in a series of repeated stimulations applied to the MFB 60 min after administration of drugs.

Statistical analysis of the effects of repeated electrical stimulation and of drugs were performed by MANOVA for repeated measures with a multivariate test of significance (Pillais statistics) or averaged test with drug treatments/anatomical structures as between-subject factor (group) and repeated electrical stimulation as within-subject factors. To simplify presentation of the results, all statistical comparisons were made for the 1st, 3rd and 5th bursts of stimulation and only data for these stimulations are presented in the figures. Two within-subject factors have been used: the first one was 'burst' (3 levels) and the second was 'stimulus' (5 levels). The individual differences between controls and the overall effect of drugs were analysed by univariate *t* tests for between-subject effects. The effects of repeated stimulation and drugs at different times after treatment were analysed by MANOVA's univariate *F*-tests with Bonferroni correction.

Chemicals and drugs

Ascorbic acid, dopamine, pargyline and α-methyl-DL-p-tyrosine-HCl were purchased from Sigma (U.S.A.), and haloperidol (Serenase) obtained from Orion Corporation (Finland). The drugs were dissolved in saline and injected intraperitoneally 10 ml kg^{-1}. Electrodes were calibrated in phosphate-buffered saline consisting of Na_2HPO_4 0.15 M and KH_2PO_4 in 0.9% w/v aqueous NaCl. The solutions were mixed in an approximately 4:1 ratio (Na:K salt) and adjusted to pH 7.4.

Results

Identification of released compounds

Pharmacological, anatomical and electrochemical studies have shown that, following electrical stimulation of the MFB in rat, dopamine is the main compound detected in striatum by means of fast *in vivo* voltammetry at the potentials used and with untreated voltammetric electrodes (Kuhr *et al.*, 1984; Millar *et al.*, 1985; Michael *et al.*,. 1987b; Suaud-Chagny *et al.*, 1989; 1992). Nevertheless, since mice were used here, we considered it prudent to provide some pharmacological and electrochemical evidence to characterize the released compound(s). Repeated electrical stimulation of the MFB evoked sharp increases in the oxidation current reflecting the release of electroactive species in the vicinity of the working electrode in the caudate and nucleus accumbens in mice. The electrochemical processes on the electrode were reversible since reduction peaks have also been found after each train of stimulation (Figure 1a). This indicated that ascorbic acid (AA) was not responsible for the peaks detected after either one or repeated trains of electrical stimulation, since AA is irreversibly oxi-

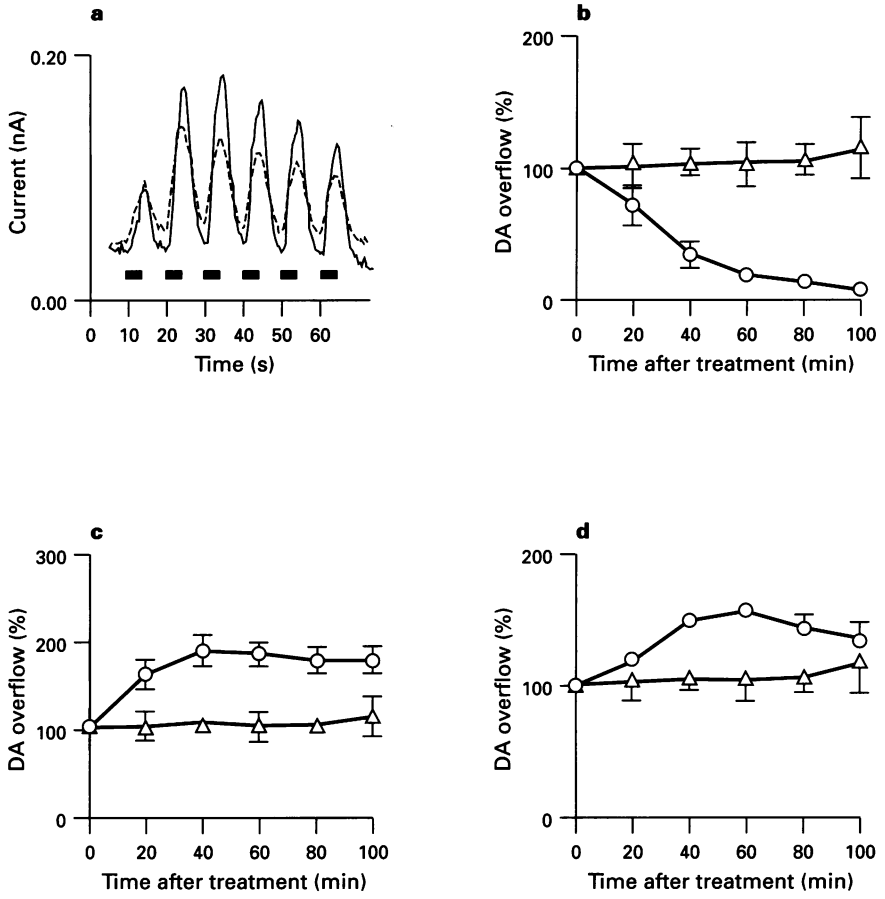

Figure 1 Electrochemical and pharmacological evidence of evoked dopamine overflow in the mouse caudate nucleus following electrical stimulation of the median forebrain bundle (MFB). (a) Original recordings from a computer screen of oxidation (solid line) and reduction (dashed line) chronoamperometric current vs. time from voltammetric electrode following 6 stimulations (4 s train duration, 50 Hz) at intervals of 10 s. The bars indicate trains of electrical stimulation of the MFB. Two traces were obtained from the same electrode and location at intervals of 40 min. Time courses of the effects of (b) α-methyl-p-tyrosine (250 mg kg^{-1}), (c) haloperidol (0.1 mg kg^{-1}) and (d) pargyline (75 mg kg^{-1}) on the evoked dopamine (DA) overflow induced by electrical stimulation of the MFB (4 s train, 50 Hz) at intervals of 20 min. Drug (○) or saline (△) was injected immediately after the first stimulation (time 0). Peak dopamine overflow is expressed as % of the peak overflow before drug administration. Each point represents the mean ± s.e.mean (vertical lines) of at least 5 mice.

dized on the electrode, thus corroborating previous results (Kuhr *et al.*, 1984). Inhibition of dopamine synthesis by AMPT at 250 mg kg^{-1} decreased the peak oxidation current (saline vs. drug-treated group, between-subject effect: $t=4.24$, $n=9$, $P=0.001$). The time course of the effects of AMPT is shown in the Figure 1b. Haloperidol at a dose of 0.1 mg kg^{-1} significantly (between-subject effect: $t=4.8$, $n=11$, $P=0.001$) increased the peak oxidation current (Figure 1c). Monoamine oxidase (MAO) inhibition by pargyline, 75 mg kg^{-1}, also increased peak current (between-subject effect: $t=1.74$, $n=11$, $P=0.01$, Figure 1d); thus, indicating that dihydroxyphenylacetic acid (DOPAC) was not contributing to the evoked overflow. This also agrees with the finding that repeated electrical stimulation of the MFB in rats caused an elevation in the basal oxidation current which reflected a slow increase in the extracellular DOPAC concentration, while the peak oxidation current induced by the short trains of stimuli was due to the dopamine (Suaud-Chagny *et al.*, 1989). These pharmacological data are, thus, in agreement with previous experiments in rats (Millar *et al.*, 1985; Michael *et al.*, 1987b; Suaud-Chagny *et al.*, 1989; 1992) and indicate that the increase in oxidation current after stimulation of the MFB in mice reflects changes in the extracellular dopamine concentration. Peak overflow of dopamine following a single stimulation (4 s train, 80 μA r.m.s. 50 Hz) reached 10.6 ± 1.4 μM ($n=11$) in the caudate nucleus and 14.9 ± 2.1 μM ($n=8$) in the nucleus accumbens, based on calibration of the electrode after the experiment.

Effects of repeated stimulation of the MFB on evoked dopamine overflow

The dynamics of dopamine overflow in the caudate nucleus induced by repeated electrical stimulation of the MFB (4 s train, 80 μA r.m.s. 50 Hz) at between-stimulation intervals of 30 and 10 s is shown in Figure 2. Stimulations at intervals of 30 s induced a gradual decline in the peak dopamine overflow. On the other hand, the dopamine decline was paradoxically less after electrical stimulation at intervals of 10 s (multivariate test: $F=5.6$, d.f. $=6$, $P=0.04$). Moreover, in this case, 4–5 stimulations after the initial one resulted in an even more pronounced dopamine overflow.

Electrical stimulation of the MFB (2 s train, 80 μA, 50 Hz) at intervals of 5 s was arranged in a 'burst' mode. Thus, 5 bursts consisting of six stimulations at intervals of 5 s were separated by 60 s between-burst intervals. This stimulation protocol allowed one to combine and investigate in a single experiment the effects of longer (60 s) and shorter (5 s) between-stimulation intervals on the dynamics of dopamine decline. This protocol also permitted a comparison of the dynamics of dopamine decline in the bursts before and after the profound dopamine depletion induced by repeated stimulation. As shown in Figures 3a and 4, the dynamics of the decline in dopamine release in the caudate nucleus in the first burst after the stimulation at intervals of 5 s were the same as after stimulation at 10 s intervals. However, the dynamics of

the decline in the following bursts were different (Figure 4, multivariate test: $F=28.5$, d.f.$=2$, $P=0.001$). The major difference was the decreased dopamine overflow after 6 repeated stimulations (except for the second stimulation) in the first burst and the increased dopamine overflow after the repeated stimulation in the following bursts, when the levels of the evoked dopamine overflow were significantly less (Figure 4, burst × stimulus interactions, averaged test: $F=13.7$, d.f.$=8$, $P=0.001$). Another difference was that dopamine overflow was less after the first stimulation in the second and following bursts than after the last stimulation of the previous bursts, even though 60 s between-burst intervals were used (vs. 5 s between-stimulation intervals in the bursts).

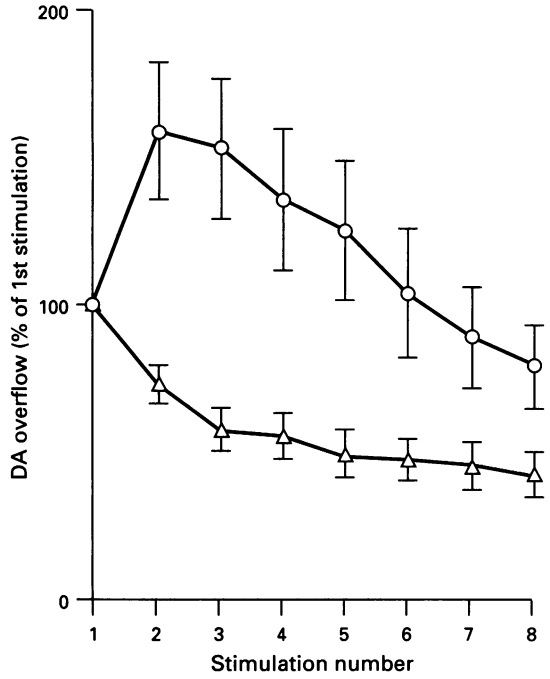

Figure 2 Dynamics of decline of evoked dopamine (DA) overflow in the mouse caudate nucleus after 8 electrical stimulations (4 s train duration, 50 Hz) of the median forebrain bundle at 10 s (○) and 30 s (△) intervals. Peak dopamine overflow is expressed as % of the peak overflow after the first stimulation in a series. Each point represents the mean ± s.e.mean (vertical lines) of 5 mice.

The same stimulation protocol was used with voltammetric electrodes inserted in the nucleus accumbens. Since uptake in the nucleas accumbens is slower than in the caudate (Missale *et al.*, 1985; Stamford *et al.*, 1988; May & Wightman, 1989, data from experiments in rats), the baseline of the bursts was more elevated than in the caudate nucleus (Figure 3b). The dopamine overflow in the 4th and 5th bursts was significantly decreased (200–500 nM) and voltammetric signals returned closer to the pre-stimulation level. The dynamics of dopamine decline in the nucleus accumbens after repeated stimulation of the MFB showed large variations. It seemed as if the results obtained in the nucleus accumbens were dependent on the sites where the electrodes were located within the structure. Thus, the mice were grouped according to relative amplitude of the dopamine overflow after the second stimulation in the first burst in comparison to the first stimulation. In 3 mice, the second peak was larger than the first one (the same results as obtained in the caudate nucleus after stimulation at intervals of 5 and 10 s) and the dynamics of dopamine overflow in the other bursts were also the same (Figure 5a). The other 3 mice showed a decrease in dopamine overflow after the first stimulation (Figure 3b) and the results of repeated stimulations significantly differed from those obtained in the caudate nucleus (Figure 5b, between-subjects effect: $F_{1,9}=26.2$, $P=0.001$). Each stimulation in the burst induced less dopamine overflow than the preceding one, and the first peak

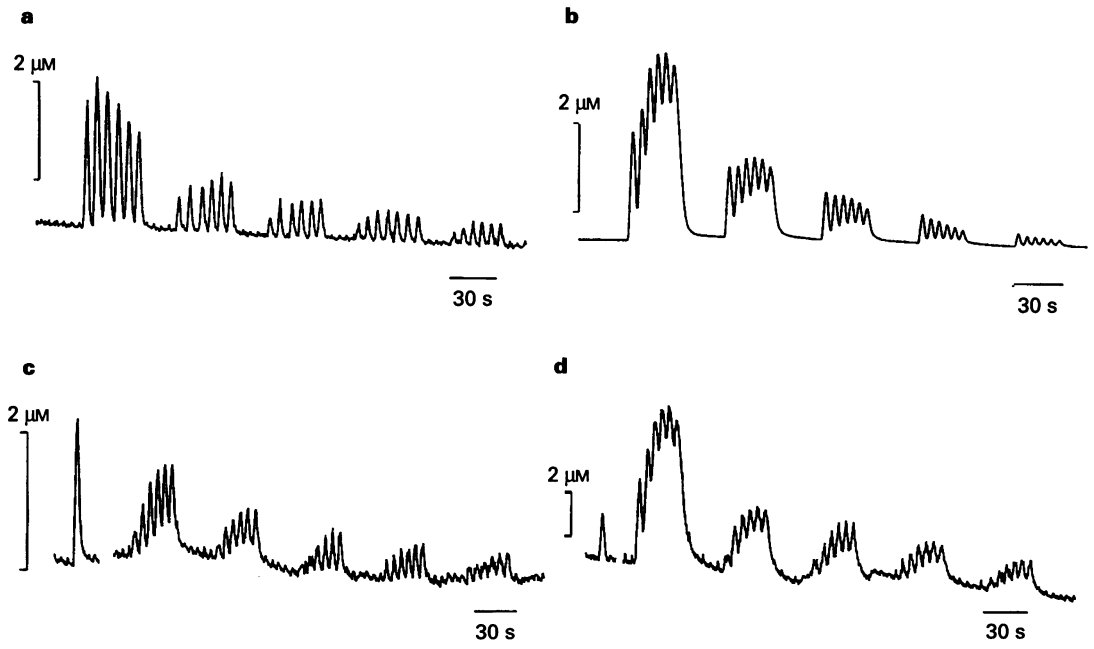

Figure 3 Original recordings from a computer screen representing changes of evoked dopamine overflow following repeated stimulation of the median forebrain bundle (MFB) organised in 5 bursts at intervals of 5 s between stimulations (2 s train duration) inside the burst and 60 s between-burst intervals. (a) Voltammetric electrode in the caudate nucleus. The trace was obtained 60 min after saline injection and corresponds to Figure 4, which represents group data. (b) Voltammetric electrode in the nucleus accumbens. Corresponding group data are shown in Figure 5b. (c and d) Voltammetric electrode in the caudate nucleus. Traces were obtained before (the first peak in the recordings) and 60 min after α-methyl-p-tyrosine 250 mg kg^{-1} (c) or haloperidol 0.1 mg kg^{-1} (d) administration and correspond to group data shown in Figure 6. Note that dopamine calibration bars are different in each recording, the time bar represents 30 s.

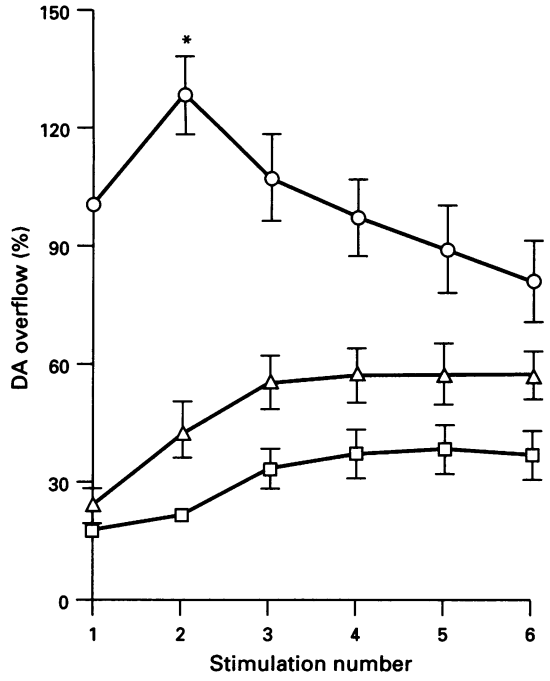

Figure 4 Changes in evoked dopamine (DA) overflow in the mouse caudate nucleus following repeated electrical stimulation of the median forebrain bundle (MFB) organised in bursts (5 bursts, 60 s intervals between bursts, 6 stimulations in bursts at intervals of 5 s, 2 train duration, 50 Hz). Only group data for the 1st (○), 3rd (△) and 5th (□) bursts are shown. Peak dopamine overflow is expressed as % of the peak overflow after the first stimulation in a series. Horizontal axis shows serial stimulations in the burst. Each point represents the mean ± s.e.mean (vertical lines) of 8 mice. When no error bars are indicated, the standard error is less than the symbol size. Note that the second stimulation in the first burst always showed larger dopamine overflow than the first stimulation ($P<0.001$, marked by asterisk).

in the burst was always larger than the last one in the preceding burst (group × burst × stimulus interactions, averaged test: $F=2.9$, d.f. $=8$, $P=0.008$).

Effects of α-methyl-p-tyrosine and haloperidol on the dynamics of the decline in the release of dopamine after repeated stimulation of the MFB

The effects of AMPT and haloperidol on the evoked DA overflow after repeated stimulation at intervals of 5 s (2 s train duration, 50 Hz) arranged in bursts, as described above, were investigated in mice with the working electrode inserted in the caudate nucleus. AMPT at a dose of 250 mg kg^{-1} significantly decreased the evoked dopamine overflow in the caudate nucleus. Thus, the peak overflow of dopamine was about 20% of the initial level 60 min after drug administration (Figure 1b). One hour after administration of the drug, repeated electrical stimulations were applied. AMPT-treated mice demonstrated (Figure 3c and 6a) a two fold increase of dopamine release after repeated stimulations in the first burst (vs. a decrease in the vehicle treated control mice) and stable dopamine release during repeated stimulations in the 5th burst (vs. increase in the controls). The differences between the overall effects of the stimulation in the 5th and 1st bursts in control and AMPT treated mice were significant (univariate F-test: $F_{1,9}=23.8$, $P=0.001$).

Haloperidol at a dose of 0.1 mg kg^{-1} caused a two fold increase in the overflow of dopamine in the caudate nucleus (Figure 1c). Repeated electrical stimulation of the MFB one hour after injections induced an elevation of the baselines in each burst (Figure 3d). When only the peak dopamine overflow was calculated (see Methods – Data presentation and statistics), a gradual decrease of the peak dopamine overflow in the first burst and a relative increase in the following peak overflows were seen (Figure 6b). Thus, qualitatively, the dynamics of dopamine depletion in control and haloperidol treated mice were the same. Quantitatively, the peak dopamine overflow after repeated stimulation in the first burst decreased to a larger extent and the relative increase in the other bursts was smaller than in the control mice (univariate F-test: $F_{1,11}=11.6$, $P=0.006$).

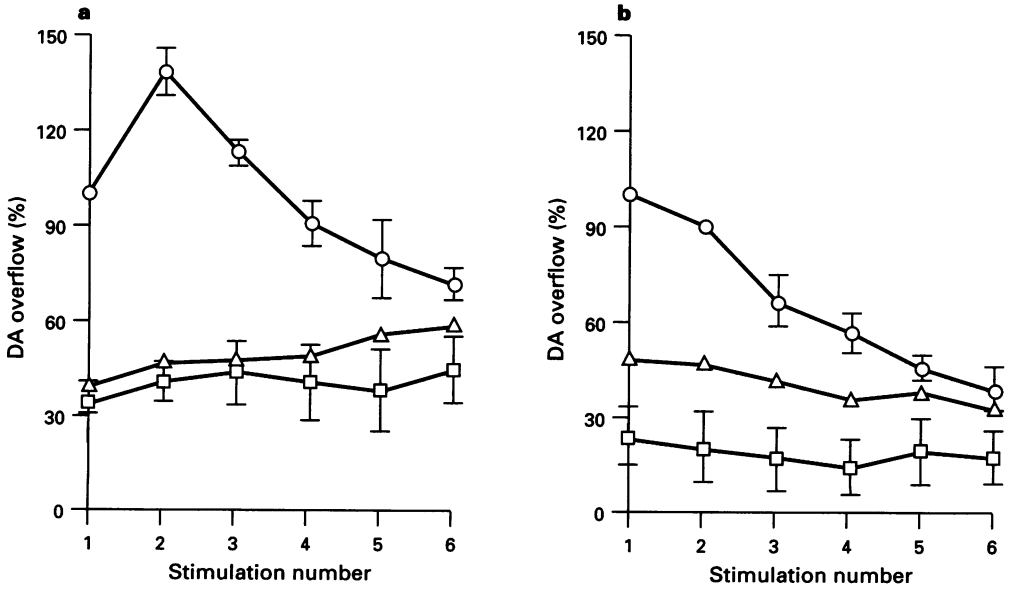

Figure 5 Changes in evoked dopamine (DA) overflow in the mouse nucleus accumbens following repeated electrical stimulation of the median forebrain bundle (MFB) organised in bursts. Stimulation protocol and representation of the results are the same as in Figure 4. Only group data for the 1st (○), 3rd (△) and 5th (□) bursts are shown. The mice were grouped according to relative amplitude of the evoked dopamine overflow after the second stimulation in the first burst in comparison to the first stimulation. (a) In 3 sites of voltammetric electrode locations the second peak was larger than the first one. (b) The other 3 sites showed a decrease in the peak overflow after the second stimulation.

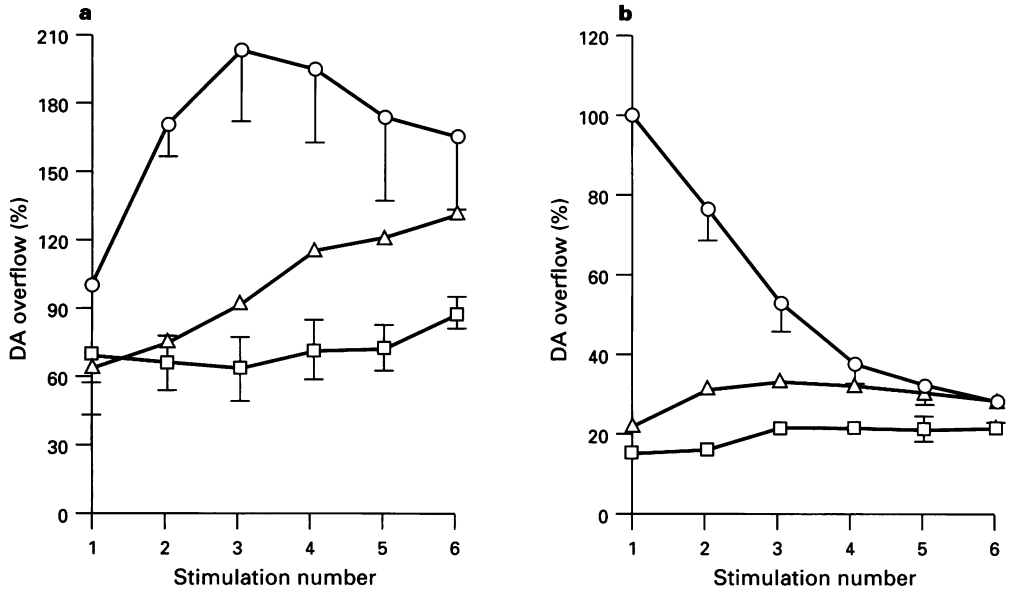

Figure 6 The effects of (a) α-methyl-p-tyrosine (AMPT) 250 mg kg^{-1} and (b) haloperidol 0.1 mg kg^{-1} on the dynamics of dopamine (DA) overflow in the mouse caudate nucleus after repeated electrical stimulation of the median forebrain bundle organised in bursts (5 bursts, 60 s intervals between bursts, 6 stimulations in the bursts at intervals of 5 s, 2 s train duration, 50 Hz). Only group data for the 1st (○), 3rd (△) and 5th (□) bursts are shown. Data were obtained 60 min after drug administration and represented as described for Figure 4. Peak dopamine overflow is expressed as % of the peak overflow following the first stimulation in a series. Note, that although the stimulation protocol here is the same as in Figure 4 data from these experiments cannot be directly compared in absolute values, due to the different reference points used. Dopamine overflow following the first stimulation in a series of bursts 60 min after AMPT or haloperidol treatment significantly differ (see time course of the effects of drugs) from the dopamine overflow following stimulation before drug administration. Using the same reference points (peak release before drug administration) would not permit a clear differentiation of data after repeated stimulation. Each point represents the mean ± s.e.mean (vertical lines) of 4 (AMPT administration) or 11 (haloperidol administration) mice.

Discussion

The main finding of this study is that repeated stimulation of the MFB at short intervals of 5 and 10 s gave rise to different patterns of evoked dopamine overflow than those seen at stimulation at intervals of 30 s. Stimulation at short intervals decreased the decline of dopamine overflow and even increased in absolute terms the evoked dopamine overflow after the first few stimulations. Thus, the duration of the interval between two consecutive stimulations is the critical factor for activation of the evoked dopamine overflow.

If each train of electrical stimulation at a given current activates only part of the ascending pathways and facilitates depolarization to the next set of stimulation to the axons located on the periphery of the stimulated area, then the succeeding stimulation will affect more axons in the MFB and will result in a greater overflow of dopamine. This could explain the results. However, for three sites of electrode location in the nucleus accumbens an increased dopamine overflow occurred but in three other sites the same repeated stimulation induced the opposite effect. Moreover, a supramaximal stimulation current which affects all axons in the MFB (Stamford *et al*, 1986) induced the same kind of increase in dopamine overflow after repeated stimulation as the routinely used stimulation current (our unpublished observation). Since activation of tyrosine hydroxylase requires at least 10 min of electrical stimulation (Murrin *et al.*, 1976), it is most unlikely that a 2 s stimulation could increase the activity of the enzyme and the amount of newly synthesized dopamine. It was suggested that short intervals between stimulations leave no time for dopamine synthesis to compensate for the decline (Michael *et al.*, 1987b). Stimulation at longer intervals (60 s between two bursts) did not evoke more dopamine overflow then stimulations at 5 s intervals (inside the burst) and AMPT had no effect on the increased dopamine overflow after repeated stimulation at short intervals. Thus, we postulate that the differences in the dopamine decline after stimulation at shorter and longer intervals was unrelated to dopamine synthesis. Since two processes – release and re-uptake – predominantly determine the evoked dopamine overflow measured by means of fast *in vivo* voltammetry (Wightman *et al.*, 1988), hypothetically a stimulation or inhibition of re-uptake after repeated stimulation, could account for the increased dopamine overflow seen in the shape of the evoked voltammetric signal. However, there were no signs of any changes of re-uptake after repeated stimulation. The lack of effect of haloperidol on the increased dopamine overflow after stimulation at short intervals showed that the involvement of the auto-receptor regulation of dopamine release was also negligible. Another possibility is that repeated stimulation increases the probability of dopamine release per pulse of stimulus, as has been suggested for transmitter release from sympathetic nerve terminals (Cunnane & Stjärne, 1984; Brock & Cunnane, 1987). Then, the question arises as to how many stimuli are needed to increase the probability of dopamine release up to its maximal value. Each 2 s of stimulation applied to MFB consisted of 100 pulses and if we accept this explanation then we should also accept that even after 3000 pulses (6 stimulations in the burst and 5 bursts) this process can still take place. We cannot totally rule out this mechanism to explain the increase in dopamine overflow. However, it does not answer the question about the origin of dopamine increase, particularly that after AMPT treatment, which dramatically decreased dopamine readily available for release.

It is possible that electrical stimulation of the MFB at intervals of 5 and 10 s activated the nonfunctional ('reserve') compartment in dopaminergic terminals. Such a phenomenon could account for both: the paradoxical increase of dopamine overflow in each burst of electrical stimulation and the effects of AMPT. From this point of view the overall dynamics of the decline of dopamine release in each burst are the sum of two separate processes: dopamine release and depletion from the

releasable pool and the translocation of dopamine from the nonfunctional storage pool. Indeed, when the releasable pool was depleted, the contribution of the nonfunctional storage pool to the increased release of dopamine was more apparent after each stimulation in the burst. AMPT decreased the amount of dopamine available for release by stimulation. This data is in agreement with the known effects of AMPT on the releasable (i.e. AMPT-sensitive) pool of dopamine in dopaminergic terminals (Ewing *et al.*, 1983). Inhibition of dopamine synthesis by AMPT did not prevent and, in fact, even highlighted the increases in evoked DA overflow after repeated stimulation at intervals of 5 s. It is known that the nonfunctional compartment of dopamine is not sensitive to AMPT (McMillen *et al.*, 1980). Thus, this compartment could donate dopamine to contribute to the increased release after repeated stimulation when the releasable pool was depleted by AMPT. Electrical stimulation of the MFB (10 s train duration, 60–100 Hz, 80–100 μA) applied at intervals of 20 min induced stable dopamine release from the releasable dopamine pool (Ewing *et al.*, 1983). Stimulation at shorter intervals induced a decline in the evoked dopamine overflow (Michael *et al.*, 1987a, b) and depletion of releasable pool in the same way as AMPT. This may explain the similar dynamics of the evoked dopamine overflow after AMPT administration and after depletion of dopamine by repeated stimulation.

In contrast to data obtained in the caudate nucleus, the characteristics of dopamine depletion from one storage pool were present in some electrode locations within the nucleus accumbens (Figure 4b). The longer the interval between stimulations of the MFB (60 s between bursts vs. 5 s between individual trains in the bursts) the less the decrease seen in the evoked dopamine release. The same results have been described for electrical stimulation of the MFB at intervals of 30 s–20 min (Michael *et al.*, 1987a,b). It is not clear, yet, whether some domains of dopamine terminals in the nuclues accumbens do not possess a nonfunctional storage pool or the between-stimulation intervals used in this study were too long to activate this pool in distinct electrode locations within the accumbens. It is also possible that a very fast equilibrium exists between two pools, eliminating the effects of stimulation at short intervals.

The term 'nonfunctional' for the AMPT-insensitive storage pool does not seem to reflect accurately the processes of dopamine storage and release, since the present results have shown that translocation of dopamine for release from this pool starts as soon as the stimulation intervals were sufficiently short. Firstly, the second peak in the burst was always larger than the first. Secondly, as indicated by the smaller increase of evoked dopamine on stimulation, haloperidol decreased the capacity of this pool, which means that dopamine also comes from it to provide greater release. Justice *et al.* (1988) have suggested that dopamine for release is replensihed from this pool if release rate exceeds synthesis rate for the releasable pool. In agreement with this suggestion, F. Gonon and his colleagues have recently shown (Chergui *et al.*, 1994) that repeated stimulation at physiological frequency, mimicking a regularly spaced activity of dopaminergic neurones (250 ms between pulse intervals), induced stable dopamine release, i.e. every pulse evoked a similar response. Repeated stimulation at intervals of 66 ms (15 Hz) which mimicked a neuronal bursting induced a facilitation of the release per pulse, similar to our results showing facilitation of the release after stimulations at short intervals.

Thus, we conclude that there are two compartments for dopamine storage simultaneously at work providing dopamine for release. Both of them contribute to the release of dopamine with each depolarizing stimulus. However, the AMPT-sensitive, releasable storage pool, which also has been associated with a cytosolic, newly synthesized pool (Besson *et al.*, 1969; Schoemaker & Nickolson, 1983) donates dopamine for the release after a single stimulation or repeated stimulations, independently of whether it is followed by another stimulation. This is not dependent on the between-stimulation intervals. The AMPT-insensitive storage pool which has been associated with vesicular storage (Schoemaker & Nickolson, 1983) is progressively activated after repeated stimulation. Between-stimulation intervals and bursting are crucial factors for the activation of this pool. The two pools might be differentiated artificially by applying short trains of electrical stimulation with intervals of less than 10 s and by depletion of intracellular dopamine by either inhibition of synthesis or repeated stimulation. However, whether the mechanism of this storage pool activation is unique for dopaminergic neurones or might be also ubiquitous for other neurotransmitter systems remains to be determined.

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