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In vivo monitoring of dopamine overflow in the central nervous system by amperometric techniques combined with carbon fibre electrodes

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Abstract

Electrochemical techniques are extensively used to investigate in vivo and in vitro events associated with neurotransmission, particularly dopamine (DA) transmission. In vivo amperometric measurements only concern evoked extracellular neurochemical events over short time periods independently of changes in basal release. In this context, DA release is evoked either by brief and low electrical or by chemical stimulation, which, respectively, mimics or elicits the physiological discharge rates of DA neurones. The combination of electrochemically treated carbon fibre electrodes with differential pulse amperometry (DPA) has been extensively used to monitor DA overflow on a 1-s time scale. The more recent in vivo combination of untreated carbon fibre electrodes with continuous amperometry gives better temporal information allowing the precise description of the kinetic parameters of the mechanisms which regulate DA overflow. The results obtained in rats extended by the results obtained in mice lacking a protein involved in DA transmission demonstrate that DA autoregulation and DA uptake: (i) contributes to the operational properties of DA terminals in converting action potentials into DA release as a high pass filter which favours short bursts of action potentials and (ii) inhibits excessive DA release which might result from prolonged and large increases in the impulse flow.

Keywords: Electrochemistry; Amperometry; Dopamine; Release; In vivo; Carbon fibre electrode; Reuptake; Autoregulation; Rat; Knockout mouse

1. Introduction

Two distinct types of neurosecretory vesicles are thought to mediate the regulated release of neurotransmitters and neuropeptides from neurones. The first type is the synaptic vesicles that store and release classical neurotransmitters such as glutamate, acetylcholine, and γ-aminobutyric acid. The second type is the secretory granules, also called large dense-core vesicles in neurones, that store and release neuropeptides. Synaptic vesicles that contain catecholamines and among them dopamine (DA) acquire an electron-opaque core after certain fixation conditions and are often referred to as small dense-core vesicles. All these organelles are likely to be present in all nerve endings, which is consistent with the view that the neuronal peptides have a modulatory role in neurotransmission. Physiological and pharmacological studies, however, indicate that exocytosis of these organelles is differentially controlled. The relative proportion of peptides and classical neurotransmitters released from the same nerve ending varies with the frequency of stimulation; a high frequency or a bursting firing activity favours peptide secretion [1,2].

The first unequivocal in vivo monitoring of DA release by electrochemical technique was accurately achieved 20 years ago. To study DA release and its regulating presynaptic mechanisms, approaches consisting in evoking DA release in vivo by electrical stimulation of nerve fibres [3,4] or by chemical stimulation of the DA cell bodies [5,6] were developed. Because the release, uptake, and autoregulation of DA occur in vivo

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in the central nervous system (CNS) on a subsecond scale, fast sensors must be used. Carbon fibre electrodes are well adapted for this purpose because of their small size, their sensitivity to DA, and their rapid time response. During the last 15 years, the combination of electrochemically treated carbon fibre electrodes with differential pulse amperometry (DPA) [4] has been extensively used to monitor DA overflow on a 1-s time scale. The more recent in vivo combination of untreated carbon fibre electrodes with continuous amperometry [7] gives better temporal information [8,9] allowing the precise description of the kinetic parameters of the mechanisms which regulate DA overflow.

2. Experimental procedure

2.1. Carbon fibre electrode

Carbon fibre electrodes have been previously described [10]. Their active part is the surface of one pyrolytic carbon fibre from 50 to 250 μm in length and 8 μm in diameter (AGT 10000; Soficar, Abidos, France). Before their implantation into the CNS and when their use is combined with DPA, carbon fibre electrodes are electrochemically treated in phosphate-buffered saline (PBS: NaCl 8 g/L, KCl 0.2 g/L, $Na_2HPO_4{\cdot}2H_2O$ 1.44 g/L, and KH_2PO_4 0.2 g/L) by applying first an anodic potential with a triangular waveform (between 0 and 2.45 V at 70 Hz for 20 s), and then two successive continuous potentials (−0.75 V then 1.5 V, for 5 s each). This treatment improves the catalytic properties of the carbon surface leading to an improved sensitivity to cations, such as DA and selectivity. Unfortunately, these improvements are paid by a slowing down of the electrode response [11].

2.2. Electrochemical techniques

2.2.1. Differential pulse amperometry

A three-electrode system is connected to a pulse voltammetric recorder (Biopulse; Radiometer Analytical, Villeurbanne, France). The auxiliary electrode (a platinum wire) and the reference electrode (a silver wire coated with AgCl) are maintained in contact with the skull by means of a semi-liquid junction. Carbon fibre electrodes are implanted into DA terminal fields such as striatum, nucleus accumbens, olfactory tubercle, and medial prefrontal cortex. Differential pulse amperometry (DPA), a technique which has high time resolution (one measurement every second) and sensitivity [5,12], is used to monitor evoked changes in extracellular DA concentration [4,5,12]. DPA is directly derived from differential normal pulse voltammetry (DNPV). It consists of an unlimited series of dual square pulses of potential. A pre-pulse is applied every second from a constant initial potential (−240 mV) to a constant final potential for 100 ms. The final potential is adjusted at +80 mV (versus the Ag/AgCl reference electrode) because DA oxidises at this potential at the surface of carbon fibre electrodes when they are electrochemically treated with a triangular potential between 0 and 2.45 V [5]. The oxidation current is differentiated during a measuring square pulse added at the end of each pre-pulse (amplitude 40 mV; duration 40 ms). Thus, this differential pulse oxidation current measured every second corresponds to the DA oxidation peak measured by DNPV. In the absence of stimulation conditions, the measured oxidation current corresponds to DOPAC (3,4-dihydroxyphenylacetic acid; a major DA metabolite) or to the basal level of DA in pargyline-treated animals. Stimulation can be achieved either by electrical stimulation of DA fibres of the medial forebrain bundle (MFB) or by pneumatic ejection of chemical compounds into the cell body areas corresponding to the DA fields explored.

2.2.2. Continuous amperometry

A reference electrode (a silver wire coated with AgCl), maintained in contact with the skull by means of a semi-liquid junction, and an untreated carbon fibre electrode are connected to an amperometric detector (AMU 130, Radiometer Analytical, Villeurbanne, France). The working electrode is held at +400 mV versus the reference electrode by the potentiostat of the detector, because DA oxidises at this potential at the surface of untreated carbon fibre electrode [7]. The current through the electrodes is continuously measured with a time constant of 1 ms. The detector output is digitised at 1 kHz without any further filtering and recorded by a MacLab/2^e system connected to a computer running the "Scope" program (ADInstruments, Castle Hill, Australia). The original recordings are often smoothed (each point is the average of several original values taken on either side of the sample point). The time interval between original values is chosen after preliminary recordings so that consecutive individual evoked responses are stable in terms of kinetics and amplitude. Because DA is not oxidised at 0 V, and to remove the transient electrical artefacts due to the stimulation, the current recorded at 0 V is subtracted to that recorded at +400 mV for each set of stimulation conditions [7].

2.3. Electrical stimulation

The ascending DA pathway (MFB) is electrically stimulated using a bipolar electrode in which both active tips (0.1 mm thick and 0.25 mm long) are parallel with a distance of 0.5 mm between them (SNEX-200, Rhodes Medical Instruments, USA). This electrode is implanted at the level of the MFB and the depth is adjusted for each experiment for the evoked DA response to be

maximal. The electrical stimulation consists of 0.3 ms current pulses with a square waveform (200–300 μA).

2.4. Local pneumatic ejection

Drugs are ejected into the DA cell body areas via of a simple [5] or double-barrelled pipette [13]. Double-barrelled pipettes consist of two lengths of glass tubing (internal diameter of 0.32 mm; calibrated at 15 mm/μl, Assistent ref. 555/5, Hecht, Sondeim-Rhoen, Germany) glued side by side, pulled together, and broken back to an external diameter of 100 μm (50 μm for each tip). The non-tapered extremity of one element is bent by heating. Just before use, each barrel of the pipette is filled, by applying negative pressure, with either a chemical compound potentially capable of changing DA firing activity or a corresponding vehicle. The final solution ejected is diluted in PBS solution as described above where $CaCl_2$ 1.3 mM is added. The pipette is implanted into the central part of the DA cell body areas such as the ventral tegmental area (VTA) and substantia nigra. The depth from the cortical surface is adjusted according to the maximal DA release evoked by chemical stimulation recorded into an ipsilateral corresponding terminal field. Ejections are performed by applying air pressure with a 1-mL syringe connected to the non-tapered extremities of the pipette by Tygon tubing. The ejected volume and the speed (usually, 65 nL/20 s) of ejection are determined under the microscope by the movement of the meniscus of the solution in the pipette. Volumes as small as 8 nL could be ejected in a reproducible way [5,13].

To visualise the track of the carbon fibre electrode in the DA terminal field explored, and that of the pipette ejection in the DA cell body area, electrolytic lesions are performed by applying, through carbon fibre electrodes, at the end of the experiment and before the removal of the brain, a continuous potential of 5 V for 5 s. For this purpose, the recording electrode remains in the brain, while the ejection pipette is carefully removed and replaced by a new carbon fibre electrode. Coronal sections (25 μm) through the DA terminal field and cell body area explored are cut with a cryostat, mounted on slides, and stained with cresyl violet. Using a light microscope, the recording sites are checked according to the atlas of Paxinos and Watson [14] for rats or that of Franklin and Paxinos [15] for mice. To show the tool placement, the photographs of the stained brain sections can be combined with the corresponding drawings adapted from the atlas (Fig. 1).

2.5. In vitro calibration

After in vivo recording, the electrode response to DA can be tested in a flow injection system similar to that described by Kristensen et al. [16]. The active part of the electrode is centred in a Teflon capillary tubing (0.8 mm in diameter, 12 cm in length), 3 mm from its end. This tubing is connected to the output of a rotary valve loop injector (Rheodyne, USA). The perfusion fluid is a PBS solution (as described above) where ascorbic acid (0.1 mM) is added. DA is dissolved in this solution and loaded in the loop injector (20 μL). The reference electrode and the auxiliary electrode, if needed, are immersed in a 50-mL reservoir, which bathes the end of the Teflon tubing. The response of the electrode depends on the flow rate of the DA solution: a higher flow rate results in a higher current according to the concept of a

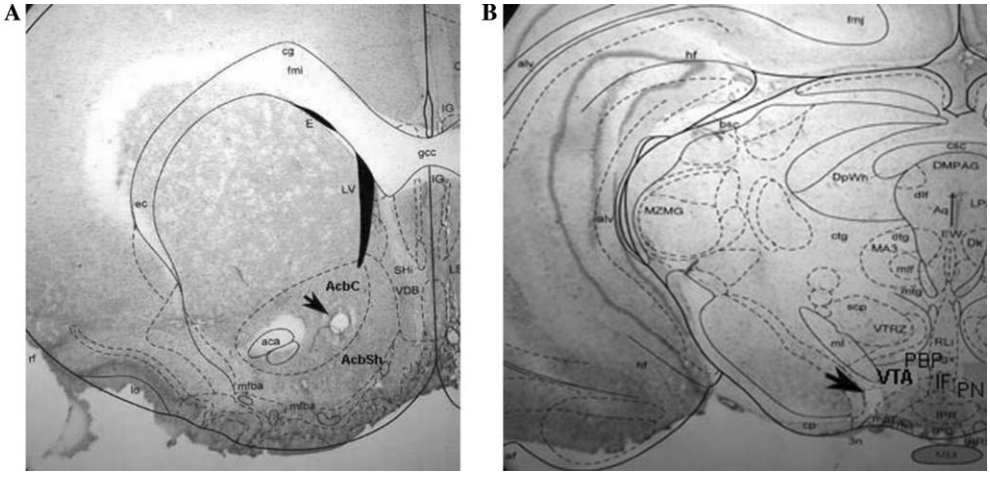

Fig. 1. Example of a localisation of the working electrode and the double-barrelled ejection pipette in the rat nucleus accumbens (Acb) (A) and the tegmental ventral area (VTA) (B). Electrolytic lesions (arrow) have been performed at the end of one experiment as described in Section 2. Figure shows the photographs of brain coronal sections (25 μm), cut with a cryostat, mounted on slides, and stained with cresyl violet, combined with the corresponding drawing adapted from the atlas of Paxinos and Watson [14]. AcbC, core part of the nucleus accumbens; AcbSh, shell part of the nucleus accumbens; PBP, parabrachial pigmented nucleus; PN, paranigral nucleus; and IF, interfascicular nucleus.

diffusion layer around the electrode. Therefore, amperometric calibration is expected to underestimate the sensitivity of the electrode. Usually, the flow rate is fixed at around 30–40 μL/s [7,17].

2.6. Data analysis

The maximal amplitude of the evoked overflow is expressed either as changes in DA concentration or as changes in oxidation current. This amplitude is also expressed as a percentage of the mean of some absolute evoked responses recorded during a control period. The DA half-life corresponds to the time to 50% decay from the point where the maximal overflow was reached.

3. Results and discussion

3.1. Methodological considerations

Electrochemical techniques are extensively used to investigate in vivo and in vitro events associated with neurotransmission, particularly DA transmission. In basal conditions, the electrochemical measurement of extracellular DA is hindered by the presence in the extracellular fluid of two other easily oxidisable compounds, i.e., ascorbic acid and DOPAC, at potentials close to the oxidation potential of DA. On the one hand, the electrochemical treatment, by improving the selectivity of the carbon fibre electrodes, allows us to resolve catechols from ascorbic acid. On the other hand, it is possible to remove the DOPAC interference by inhibiting its synthesis thanks to a pharmacological treatment with pargyline. However, brief stimulation of the DA impulse flow evokes an immediate and short-lasting increase in the oxidation current followed by a rapid recovery. This increase is solely due to the oxidation of DA released by the stimulation. If the stimulation is brief, DOPAC does not contribute to this oxidation current since its half-life is about 10 min.

In vivo amperometric measurements combined with carbon fibre electrodes only concern evoked extracellular neurochemical events over short time periods independently of changes in basal release. The release is evoked by brief stimulations close to physiological ones. Thus, these approaches combine the advantages of an in vivo study in terms of physiological relevance with those of an isolated model as precise as in vitro brain slice. In these conditions, the DA overflow recorded results from DA release minus DA clearance by reuptake.

The in vivo electrochemical techniques vary in sensitivity, chemical resolution, and temporal resolution. Amperometry exhibits excellent time resolution. Because of its modest chemical resolution, the nature of the chemical compound measured must be confirmed by electrochemical, anatomical, pharmacological, and physiological evidence. The electrode potential must correspond to the oxidative potential of the compound determined in vitro and in vivo by selective techniques such as voltammetry. Anatomical specificity is provided by the locations of both recording electrode and stimulating tool (Fig. 1). Pharmacological evidence lies in predicted responses induced by specific drugs affecting storage, release and reuptake, and acting on receptors. Physiological evidence is based on neurotransmission criteria.

Differential pulse amperometry, with a measurement every second, gives access to the DA overflow evoked by brief and low electrical or chemical stimulation, applied often for 20 s, which, respectively, mimics or elicits the physiological discharge rates of DA neurones.

Continuous amperometry combined with untreated carbon fibre electrode provides the best time resolution [8,9], close to the electrophysiological one, which makes new observations possible. First, this approach can record the very brief DA overflow evoked by a single pulse of stimulation without any pharmacological treatment allowing us to correlate DA overflow with electrophysiological data [7–18]. Second, it enables us to accurately measure the rate of DA elimination, the release of which is evoked either by a single pulse or by brief electrical stimulations closely mimicking naturally occurring bursts of action potentials [19–21]. Third, it is useful to precisely determine the time course of autoinhibition, especially its onset [17,22].

The first results and hypotheses have been more recently extended and the kinetics more precisely described thanks to the use of amperometric techniques in mice lacking a protein involved in DA transmission [17,20].

The in vitro calibration is mainly used to compare the amplitude of the evoked overflow between groups of animals. Given the differences between in vitro and in vivo conditions, the absolute value of evoked changes in DA concentration must be considered as a rough estimate.

3.2. Relationship between excitation and DA efflux

Dopaminergic neurones discharge either in a single spike mode with a mean firing rate of about 4–5 Hz (i.e. with a mean inter-spike interval of 250 ms) or in a bursting pattern [23–25]. Usually bursts consist of two to six spikes at 15 Hz (i.e. with a mean inter-spike interval of 70 ms). These neurones can switch from one discharge pattern to the other while the mean discharge rate remains little affected [26]. Physiological stimulation such as sensory and appetitive stimuli favours bursting activity [26–29]. Electrophysiological studies have pointed out the importance to consider, beyond the changes in the mean discharge rate of the DA neurones, the changes in the discharge patterns evoked by physi-

ological and pharmacological stimulations [30,31]. Numerous studies, mainly achieved by in vivo DPA, have shown the two firing activities evoke neurochemical responses, which may have physiological and physiopathological consequences. The bursting pattern is actually twice as potent as regularly spaced activity in inducing DA overflow. This was first demonstrated by the measurement of the DA efflux evoked by electrical stimulation of the ascending DA pathway mimicking the both DA patterns [4,18]. According to the regulation of the firing rate and the burst firing of DA neurones by glutamate afferents into the VTA, glutamatergic agonists, such as *N*-methyl-D-aspartate (NMDA) and quisqualate, pressure-ejected near DA neurones, enhance DA firing activity [5]. When the mean firing rate is considered NMDA is as potent as quisqualate, but NMDA promotes burst firing while quisqualate induces a sustained activity. As regards DA release, NMDA (Fig. 2) is twice as potent as quisqualate.

In the same way, it has been shown that neurotensin ejection into the VTA, whatever the concentration used, elicits a burst firing mode separated by a post-burst inhibitory period of variable duration. In these conditions, the evoked DA overflow is related to the resulting effect on the mean firing rate. Thus, short interburst intervals permit an increase in mean firing rate leading to an increase in DA overflow (Fig. 2). Long interburst intervals, showing an overexcitation which could be the primary factor leading to the depolarisation block of DA neurones, induce a decrease in mean firing rate leading to a decrease in DA overflow [32].

Taken together these results show that DA terminals convert physiological impulse flow into DA release as a high pass filter which favours bursts of action potentials and further underline the importance of the bursting pattern in DA transmission.

The improved time resolution of the continuous amperometry combined with untreated carbon fibre electrodes allowed us to show that during electrical stimulation mimicking the regular spaced mode (i.e., around 4–5 Hz), the released DA is readily eliminated between every pulse by neuronal reuptake (within 200 ms in rats) [7,18]. Consequently, DA does not accumulate in the extracellular fluid (Fig. 3). Thus, the maximal amplitude of the overflow evoked at 4 Hz does not depend on the train length but corresponds to the amplitude overflow evoked by every individual pulse. On the other hand, only a small facilitation of the release per pulse is observed during stimulation mimicking a burst, i.e., the DA released per pulse remains approximately constant during a train [18]. Consequently, the high extracellular DA level evoked by a burst is actually mainly due to accumulation of the released DA (see below). These results have been confirmed from mice lacking DA transporter. In these mice, "the DA overflows evoked by four pulse stimulation at either frequency were almost identical." Consequently, a "burst was not able to evoke a significant increase in the extracellular DA over the basal level [20]."

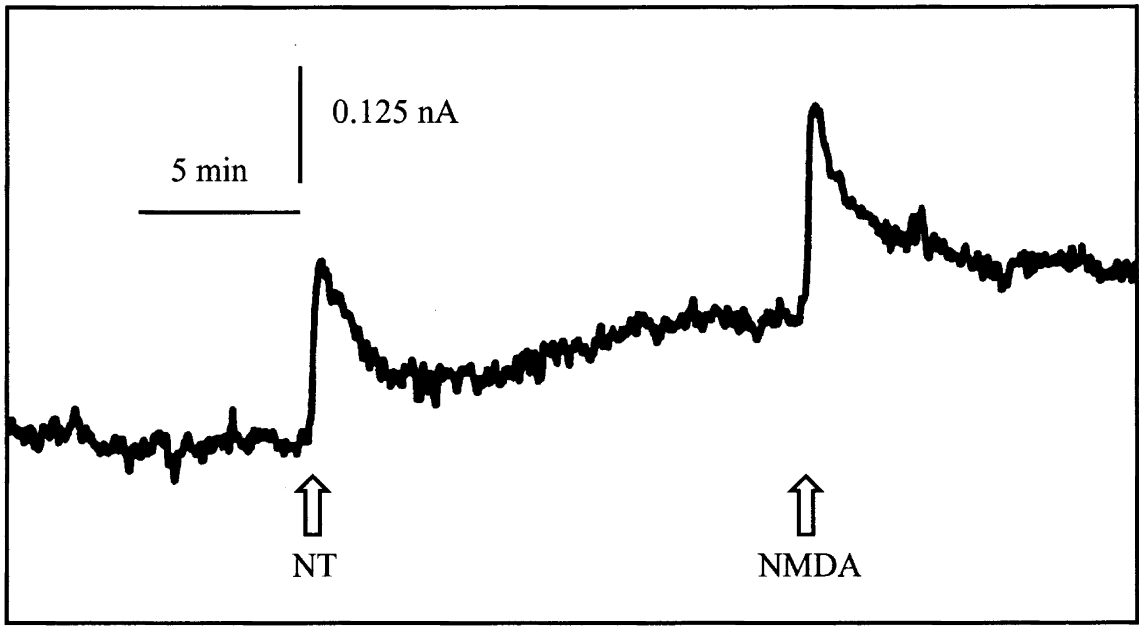

Fig. 2. Typical recording of the effect of local administration of *N*-methyl-D-aspartate (NMDA, 10^{-4} M in 65 nL) and neurotensin (NT, 10^{-7} M in 65 nL) into the ventral and tegmental area on the DA overflow evoked in the nucleus accumbens of anaesthetised mice. NMDA and NT were alternatively pressure ejected every 15 min through a double-barrelled pipette. The differential pulse oxidation current appearing at +85 mV and corresponding to DA overflow was monitored every second by DPA combined with electrochemically treated carbon fibre electrode.

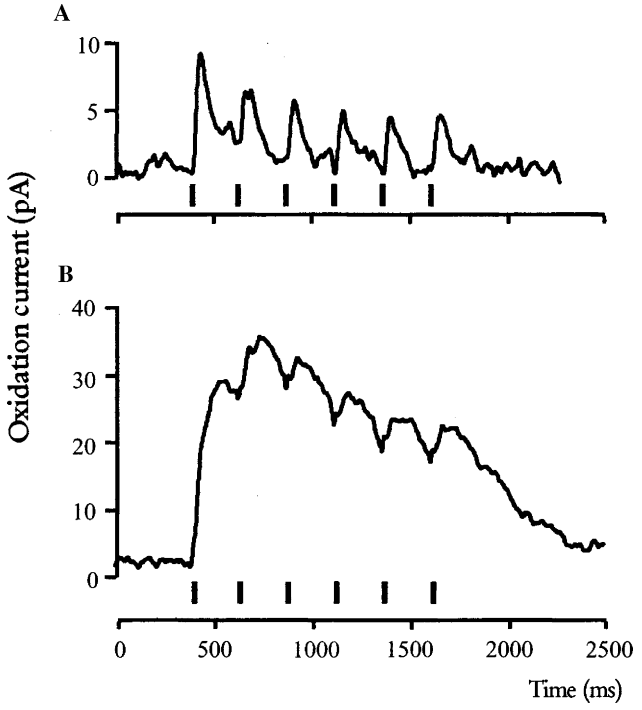

Fig. 3. Effect of uptake blocker (nomifensine, 10 mg/kg, i.p.) on DA overflow evoked in the striatum of anaesthetised rat by electrical stimulation of the ascending DA pathway. The oxidation current was continuously monitoring at +400 and 0 mV by continuous amperometry combined with untreated carbon fibre electrode. Stimulation trains composed of 6 pulses (0.5 ms, 300 μA) at 4 Hz were applied every 15 s for 70 min. In control conditions, each pulse evokes transient increase in DA overflow readily eliminated between every pulse by neuronal reuptake (within 200 ms in rats). Consequently, DA does not accumulate in the extracellular fluid. The DA blocker induces a slowing down of DA elimination. As a result, the DA release evoked by each pulse could accumulate with that evoked by the other pulses leading to an enhanced overall evoked DA. The figure shows the responses calculated by subtracting the original trace measured at 0 mV from that measured at +400 mV just before and 30 min after drug injection. Each original trace corresponded to the average of 20 successive responses evoked every 15 s.

As regards the physiology of the DA systems it is proposed that the regular spaced activity would correspond to the tonic activity of the system responsible for the basal level of DA in the extracellular fluid determining the baseline level of DA receptor stimulation. Changes in tonic activity should elicit homeostatic compensations capable of modulating the DA efflux or the postsynaptic response evoked by phasic (bursting) activity or both. The phasic DA efflux is proposed to consist of a large transient increase in DA release that stimulates postsynaptic DA receptors and to correspond to a significant signal in response to physiological stimulation such as sensory and appetitive stimuli [26–29].

"Current models into the pathophysiology of schizophrenia suggest that this disorder is not due to a primary pathology within the DA system but is subjected to a dysregulation as a consequence of the abnormal cortical glutamatergic afferents [33]." Thus, Grace [34] proposed that in schizophrenia the tonic DA levels would be decreased resulting in a decreased activation of DA receptors. The decrease in tonic DA being maintained for an extended period of time, further compensatory processes would be activated leading to a presynaptical down-regulation and an postsynaptical up-regulation. In this context, the phasic DA response would be emphasised in terms of the amplitude of the DA overflow and postsynaptic response. This hypothesis underlines the importance to consider, beyond the changes in basal extracellular DA level, the changes in tonic versus phasic DA release.

3.3. Influence of autoregulation on DA efflux

It is largely admitted that DA autoregulation controls the amplitude of the DA release via a local feedback mediated by presynaptic autoreceptors of the D2 type, which regulate DA synthesis and secretion. Thus, activation of DA autoreceptors decreases DA release, while DA antagonists increase it. In vivo DPA and continuous amperometry combined with carbon fibre electrode have largely contributed to precisely describe this mechanism in conditions close to physiological ones [22,35]. Many results obtained in rats have been confirmed and extended by data obtained in mice lacking D2 receptors [17].

In contrast to the findings obtained from in vitro studies, in vivo continuous amperometry allowed us to show that D2 antagonists could enhance the DA overflow evoked by a single pulse or by the first pulse of a stimulation train. Thus, the in vivo data support the view that the basal extracellular level supplied by tonic activity is high enough to activate DA autoreceptor and tonically inhibit subsequent DA release [22]. A more marked autoinhibition appears with repetitive stimulation pulses, the DA release evoked by each pulse inducing inhibition of the further release. The relationship between the DA overflow evoked by repetitive stimulations and autoinhibition amplitude appears complex since the strength of autoreceptor stimulation depends on the extracellular DA level and the duration of the autoreceptor exposure to DA. On the one hand, pharmacological and physiological data have proved that the strength of autoregulation is positively correlated with the extracellular DA level. For example, the potency of DA antagonists on evoked DA overflow is positively correlated with the stimulating frequency (in a physiological range) while the potency of DA agonists is inversely correlated [36]. On the other hand, kinetic studies have demonstrated that autoinhibition is maximal between 150 and 300 ms after stimulation and ineffective 1 s after [17]. Consistent with these results autoinhibition plays a role in the regulation of the DA release evoked by electrical stimulation mimicking tonic activity (4 Hz) [22] and bursting activity (6 pulses,

15 Hz). The largest autoregulation driving strength operates on the DA overflow evoked by the late phase of the bursting activity [17]. Thus, autoregulation contributes to the operational properties of DA terminals in converting action potentials into DA release as a high pass filter which favours short bursts of action potentials and inhibits excessive DA release which might result from prolonged and large increases in the impulse flow.

3.4. Relationship between DA efflux and DA elimination

Reuptake into DA terminals, achieved by the DA transporter (DAT), is the main mechanism responsible for the clearance of the released DA. Amperometric studies have clearly shown that pharmacological inhibition of the DAT slows down the DA elimination and consequently prolongs the DA half-life and enhances the extracellular DA level (Fig. 3). The DA half-life is enhanced by one order of magnitude after pharmacological inhibition [19] and by roughly two orders of magnitude in mice lacking DAT [20]. When results obtained from the striatum of naive rats are simulated with a specific mathematical model, the kinetic parameters for DA uptake, i.e., V_{max}, K_m, and [DA]p (amount of instantaneous DA concentration change per stimulation pulse), can be estimated. Change in stimulation frequency does not affect K_m (0.23 μM) and [DA]p (0.29 μM), while V_{max} slightly increases as the frequency increases [21]. These results have been confirmed in mice lacking DAT. In these animals, the released DA accumulates in the extracellular space with successive pulses involved in a brief train whatever the stimulation frequency used in a physiological range. As a consequence, the overflows evoked by 4 pulses at 4 and 15 Hz exhibit the same amplitude [20].

These studies underline the major role played by the reuptake in regulating the relationship between impulse flow and DA release. In standard animals, the large excess of DA overflow evoked by a brief burst activity compared to that observed with single pulse activity is due to the extracellular accumulation of the released DA as a result of reuptake overcoming. Moreover, equilibrium between release and uptake is responsible for the plateau reached after a few pulses by the DA overflow evoked by train stimulation. Thus, DA uptake contributes to the operational properties of DA terminals in converting action potentials into DA release as a high pass filter which favours short bursts of action potentials and to limit excessive DA release which might result from prolonged and large increases in the impulse flow.

4. Concluding remarks

Amperometric techniques combined with carbon fibre electrodes, and particularly continuous amperometry, thanks to its excellent time resolution, have proved their efficiency to precisely describe in vivo the mechanisms regulating DA release in conditions close to physiological ones. They give access to the parameters governing the efficiency of the DA transmission, which can be specifically altered in neurologic and psychiatric disorders and by pharmacological treatments. Other electrochemical techniques, such as high speed chronoamperometry and fast-scan cyclic voltammetry, providing lower temporal resolution but higher chemical resolution, can be also helpful to investigate the rapid events associated with DA transmission [8]. Microdialysis combined with HPLC or capillary electrophoresis gives the safest chemical information and is still the more reliable technique to monitor variations in the basal extracellular DA level.

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