Monitoring of Potassium-stimulated Catecholamine Changes in Striatal Synaptosomal Preparations and in Corpus striatum of Rats: A Comparative Voltammetric Study

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Abstract. Voltammetric techniques were used to compare the effects of K^+ -induced depolarization on catecholamine levels in *in vitro* synaptosomal preparations of the corpus striatum with those in the *in vivo* corpus striatum of anaesthetized animals. *In vitro*, the catechol-oxidation currents could be recorded only in dopamine-preloaded synaptosomes . In isolated synaptosomes prepared in the presence of elevated concentrations of Ca^{2+} (1 mmol.l^{-1}) and Na^+ (135 mmol.l^{-1}), K^+-induced depolarization had variable effects on catechol-oxidation current. The stimulatory effect of K^+-induced depolarization (a short transient increase of catechol-oxidation current lasting for 30 s) could be observed after the addition of dopamine loaded synaptosomes in EGTA into the medium with elevated K^+ concentration (90 mmol.l^{-1}) and decreased concentrations of Na^+ (75 mmol.l^{-1}) and Ca^{2+} (0.75 mmol.l^{-1}). These results suggest that experimental procedures and parameters of ionic composition of incubation media have to be carefully controlled, owing to an enhanced *in vitro* permeability of membranes of isolated synaptosomes for Ca^{2+} and Na^+. In *in vivo* experiments, microinjection of KCl (3 μl of 0.5 mol.l^{-1} KCl in 10 mmol.l^{-1} HEPES, pH 7.4) resulted in the appearance of several phases of catechol-oxidation current: the current increased (to severalfold of the control values) followed by a decrease or even total disappearance, with a gradual return to control values. Under conditions of depletion of extracellular calcium by EGTA (5 μl of 0.5 mol.l^{-1} KCl + 0.25 mol.l^{-1} EGTA in 10 mmol.l^{-1} HEPES, pH 7.4) K^+-induced depolarization confirmed the key role of calcium in the release of catecholamine transmitters as well as that in processes regulating the uptake and metabolism of these transmitters. The voltammetric techniques used in the

present study may be a useful tool in extending of our knowledge about the cellular mechanisms of stimulus-response coupling in nerve cells.

Key words: Catecholamine — Potassium — Calcium — Rat striatum — Synaptosomes.

Introduction

The rise of intraneuronal free Ca^{2+} concentration plays a key role in the release of neurotransmitters from nerve terminals (Katz 1969; Katz and Miledi 1969; Miledi 1973). Chemical stimulation by elevated K^+ levels in extracellular environment is most frequently being used as an activator of processes connected with the increase of intraneuronal Ca^{2+} concentration due to Ca^{2+} entry or its release from internal stores (Blaustein 1975; Nachshen and Blaustein 1980; Duncan 1983; Akerman and Nicholls 1983; Adam-Vizi and Ashley 1987; Okada et al.1989).

In situ the mammalian nervous system allows but limited methodological approaches to be employed, due to the highly complex nature of the system. Owing to this, the majority of data concerning the coupling of K^+-induced membrane depolarization with transmitter release come from *in vitro* experiments on isolated nerve endings, using fluorimetric and radionuclide methods (Blaustein 1975; Blaustein et al. 1978; Adam-Vizi and Ligeti 1986; Chandler and Leslie 1989; Okada et al. 1989; Nicholls 1989). Although isolated nerve endings have proven a suitable experimental model, the results obtained so far have pointed to a number of limitations concerning the interpretation, arising from the chemical methods and experimental conditions employed, which substantially differ from those encountered in vivo (Orlický and Varečka 1990).

In the present work K^+ depolarization-induced changes in in vitro levels of electroactive substances in the environment of isolated nerve endings from rat corpus striatum were compared with those measured voltammetrically *in vivo* in the extracellular space of the rat brain corpus striatum.

Materials and Methods

Chemicals

Saccharose, HEPES, $MgCl_2$, $CaCl_2$ and EGTA were obtained from MERCK (Darmstadt, FRG), DOPAMINE, Pargyline were from Sigma (USA). Other chemicals of analytical grade were from Lachema (Brno, Czechoslovakia).

Experimental media

a) Isolation medium (IM) contained (in $mmol.l^{-1}$): saccharose 320, EGTA 1, EDTA 1, HEPES 10, pH 7.4.

b) Basic physiological medium (KRM) contained (in mmol.l^{-1}): NaCl 135, KCl 5, $MgCl_2$ 1.2, EGTA 1, glucose 10, HEPES 10, pH 7.4, with or without 1 $CaCl_2$. Depolarization medium was of the same composition as KRM the only difference being isomolar replacement of NaCl with KCl.

Experimental procedures

In vitro experiments.

Isolated synaptosomes were prepared according to Gray and Whittaker (1962) from isolated striata of 5 - 6 anesthetized Wistar rats (body weight 150 - 200 g, Dobrá Voda). Following separation on a saccharose gradient, the synaptosomes were resuspended in threefold volume of the isolation medium and centrifuged at 17,000×*g* for 30 min. The synaptosomes containing sediment was resuspended twice in the basic medium (KRM-EGTA) and repeatedly centrifuged. The synaptosome suspension (10 - 15 mg protein/ml) was stored on ice until used.

For voltammetric measurements of catechol-oxidation currents (CA.OC), the synaptosomes were kept in a microthermostat apparatus for voltammetry (Fig. 1,*B*) as described elsewhere (Murgaš and Padúch 1989). The apparatus allows to keep the temperature of 1 ml solution at 37 °C under continuous saturation with oxygen and stirring. The three voltammetric electrodes were inserted in channels specially adjusted to hold them. The working electrode (W) was a varnish-insulated graphite rod, 0.5 mm in diameter. The active face was dressed mechanically (Murgaš et al. 1989) and electrochemically (Mermet and Gonon 1986; 1988). The reference Ag/AgCl electrode was of the common type (Bureš et al. 1967).

In vivo experiments.

Male Wistar rats, weighing 300 - 350 g were used. Thirty min after chloralhydrate anesthesia (400 mg/kg, i.p.), the animals were fixed in a stereotactic apparatus. A hole was drilled into the skull bone, the dura and the pia mater were perforated, and three voltammetric electrodes were inserted (Fig. 2,*A*).

The carbon working microelectrode (W) contained 5-8 pyrolytic fibres (each approx. 7 μm in diameter) inserted into a glass micropipette with an exposed tip length of 200 μm. The auxiliary (A) electrode was a minute watch screw of inox steel screwed into the skull bone, the reference (R) electrode of Ag/AgCl wire was in contact with the dura mater.

The three-electrode polarographic analyzer (PA4, Lab. potřeby, Prague)(Fig. 1,*A*) worked in differential pulse voltammetry mode (scan rate 50 mV/s, pulse amplitude 50 mV, pulse duration 60 ms, pulse period 0.2 s). Changes of CA.OC were read from curves recorded by an XY plotter.

A glass micropipette (tip diameter approx. 100 μm) allowing dosage in microliters was used to microinject drugs into the brain (pressure application)(Pavlásek and Dekan 1986).

Stereotactic atlas (Fifková and Maršala 1960) was used for orientation during the impalement of the working electrode and the micropipette and during the withdrawal of material for synaptosome preparation.

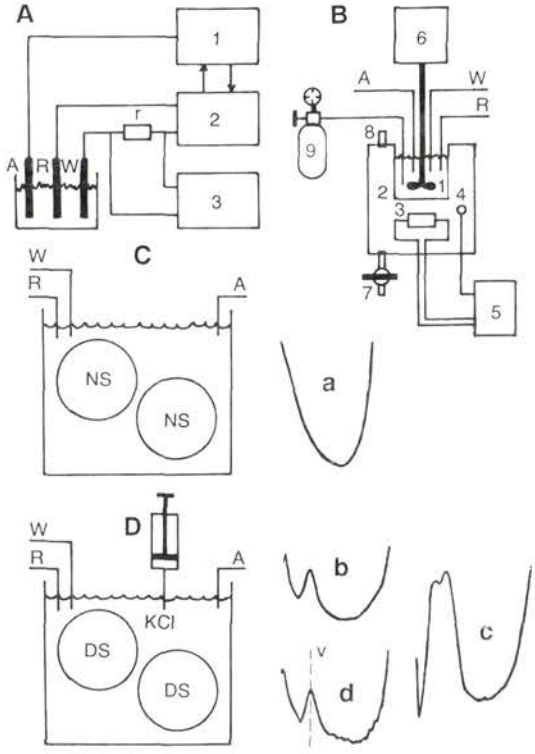

Figure 1. Voltammetry *in vitro*: measurements of CA.OC in a synaptosome suspension. A. The principle of voltammetry in three-electrode-layout: W- working electrode; R - reference electrode; A - auxiliary electrode. 1 - polarization voltage control, 2 - polarization voltage generator, 3 - current recording on resistor r. B. Schematic representation of the microincubator for voltammetry (patent pending). 1 - working chamber with incubation medium, 2 - heated space , 3 - heating element, 4 - thermistor, 5 - thermostat, 6 - stirring appliance, 7 - filling and outlet valve, 8 - aeration slot, 9 - bomb containing 95 % O_2 + 5 % CO_2, with reduction valve. Electrodes A,R,W, see under A. C. Working chamber filled with a suspension of native synaptosomes (NS) from corpus striatum in EGTA incubation medium. Electrodes A,R,W, see under A. a - voltammetric record with no signs of oxidoreduction (no peak). D. Working chamber filled with 1 ml KRM incubation medium containing 75 mmol.l^{-1} NaCl + 75 mmol.l^{-1} KCl + 1 mmol.l^{-1} $CaCl_2$ and 0.25 ml of a suspension of dopamine loaded synaptosomes (DS) in KRM-EGTA medium (time t). Following a control recording - b (recording started at time t + 30 s), 50 μl of 0.5 mol.l^{-1} KCl in 10 mmol.l^{-1} HEPES (pH 7.4) was added into the chamber (time t + 60 s), and records c (recording started at t + 70 s) and d (start at t + 120 s) were taken. The broken line in d represents voltage on the working electrode (v = +50 mV) at which the peak appeared. Electrodes A,R,W, see under A.

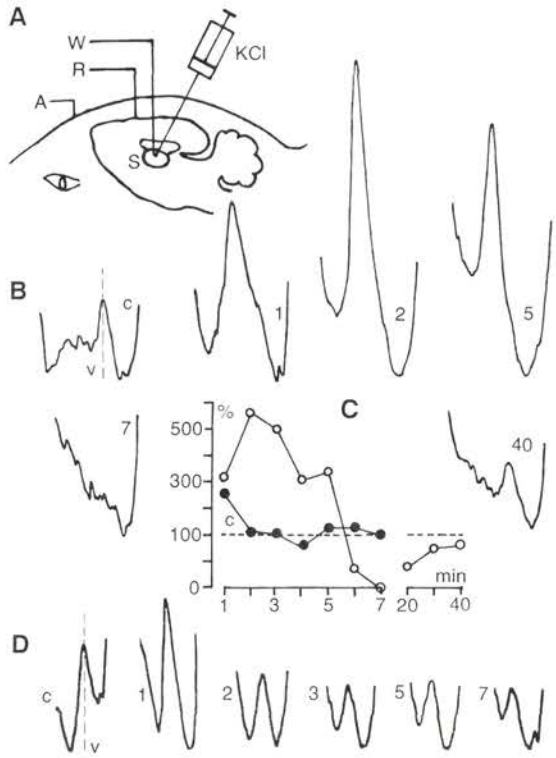

Figure 2. Voltammetry *in vivo*: measurements of CA.OC in rat brain. *A*. Experimental layout: electrodes - W, working impaled in corpus striatum (S); R, reference; A, auxiliary. After taking control records, application micropipette with KCl was impaled into the proximity of the tip of W. *B*. A series of measurements in a single experiment. c - control recording. The broken line represents the voltage on the working electrode (v = +370 mV) at which maximum peak amplitude was recorded. 1,2,5,7,40 - records taken at the time intervals indicated (min) after microinjection of 3 μl of 0.5 mol.l^{-1} KCl in 10 mmol.l^{-1} HEPES (pH 7.4) into S. *C*. Changes of peak amplitude (ordinate: % of control values) in time since KCl administration (abscissa: min). Results of 2 experiments: empty circles, experiment illustrated in B; filled circles, experiment like in D; amplitude measured according to the length of the peak descending arm. *D*. A series of measurements in a single experiment. c - control recording (v = +390 mV). 1,2,3,5,7 - records starting at the intervals indicated (min) after microinjection (5 μl) of 0.5 mol.l^{-1} KCl + 0.25 mol.l^{-1} EGTA in 10 mmol.l^{-1} HEPES (pH 7.4) into S.

Results

In vitro *experiments*

Within the voltage range applied on the working electrode (−150mV − +800mV), no CA.OC(peak) suggesting oxidoreduction reaction was recorded in control experiments in EGTA incubation medium. Additions into the measuring chamber of KCl and/or $CaCl_2$ had no effect in this respect. Similarly, no signal pointing to the presence of an electroactive agent (Fig. 1*a*) could be detected with suspension of native synaptosomes (NS) in EGTA medium in the chamber (Fig. 1*C*), or with dopamine + pargyline (20 μmol.l^{-1}) loaded, centrifuged, and EGTA medium suspended synaptosomes (DS) in the measuring chamber. No signal appeared even after damaging the NS membranes with a detergent (Triton X-100, 0.1 mg/ml). When synaptosome suspension in EGTA medium was loaded in the measuring chamber, peak appeared with an amplitude maximum ranging between +40 and +120 mV (50 ± 27 mV, arithmetic mean ± SD of 11 samples; Fig. 1,*b*,*d*). Peaks appeared at these voltages also after dopamine (DA) application into the EGTA medium in the chamber in the absence of synaptosomes; upon increasing dopamine concentration in the chamber (concentrations tested 1–5 μmol.l^{-1}) the peak amplitude grew and the peak was shifted to the left (towards lower voltages on the working electrode). Repeated measurements (in regular 1 min intervals) in a DS sample over 10–20 min showed a gradual decrease of the peak amplitude.

The testing of the effects of DS environment variation included variation of the sequence of KCl and $CaCl_2$ application into the measuring chamber. Addition of 50–200 μl 0.5 mol.l^{-1} KCl in 10 mmol.l^{-1} HEPES (pH 7.4) into the KRM - EGTA medium containing DS (final KCl concentration in the chamber 0.03–0.12 mol.l^{-1}) induced no significant change in the peak amplitude (results of 3–5 measurements made in each of 6 samples, 1 min after KCl addition). $CaCl_2$ (5 - 20 μl of 0.25 mol.l^{-1} $CaCl_2$ in 10 mmol.l^{-1} HEPES, pH 7.4) addition into the EGTA medium with DS (final $CaCl_2$ concentration in the chamber 0.2–5 mmol.l^{-1}) resulted in statistically significant reduction of the peak amplitude in 5 out of 6 samples. If $CaCl_2$ (20 μl) was applied followed by 100 μl KCl, the peak decreased following $CaCl_2$, whereas no changes were observed following KCl (results of measurements of 4 samples). When KCl application preceded that of $CaCl_2$, the effect of $CaCl_2$ was not uniform: the peak amplitude increased in 3 out of 6 samples, and decreased in the remaining cases. This result suggested that a voltammetrically detectable increase of the electroactive agent concentration in EGTA medium may appear due to the effect of $CaCl_2$ on KCl-predepolarized DS. Obviously, this effect depends on the actual concentration of free Ca^{2+} following $CaCl_2$ reaction with EGTA. The following experiments were designed to test the above suggestion. The chamber was filled with 0.75 ml incubation medium containing 75 mmol.l^{-1} NaCl + 75

mmol.l^{-1} KCl + 1 mmol.l^{-1} $CaCl_2$, and 0.25 ml DS suspension in KRM - EGTA medium was added into this environment. The recorded peak (recording started in 30th s after DS addition, Fig. 1,*b*) substantially increased following addition of 50 μl KCl (0.5 mol.l^{-1}) into the chamber (Fig. 1,*c*, the recording started in 10th s after KCl addition and in 70th s after DS addition into the chamber). The increase was of short duration as at subsequent measurement (starting in 30th s after the completion of the previous measurement) the peak (Fig. 1,*d*) was comparable with that obtained during the first recording (Fig. 1,*b*). Similar results obtained from 4 different samples confirmed that, in incubation medium containing KCl-predepolarized DS synaptosomes and $CaCl_2$, K^+-induced depolarization (elevated KCl concentration in the medium) raises the concentration of the electroactive substance. This increase is of short duration and is voltammetrically detectable.

In vivo *experiments*

Within the range of voltages applied onto the working electrode (−150 mV – +800 mV), the CA.OC peak recorded from corpus striatum (0.5 mm rostrally from the bregma, 1.5–2 mm laterally from the medial line, 3.5–4 mm below the brain surface) showed a maximum at +350– +400 mV (380 ± 18 mV, mean of 7 experiments) (Fig. 2,*B*,*c*). During the series of measurements performed within each experiment (interval 1 min), as soon as control recordings with constant pattern had been obtained, the tip of the application micropipette was impaled (with the aid of a micromanipulator) close to the site of the working microelectrode (the distance between the site of measurement and that of application < 1 mm), and 3 μl of 0.5 mol.l^{-1} KCl in 10 mmol.l^{-1} HEPES (pH 7.4) were injected (Fig. 2,*A*). As early as within the first minute after the application, the peak grew by several hundreds percent (Fig. 2*B*,1); in several cases, this growth continued further during the second minute (Fig. 2*B*,2, Fig. 2,*C*, empty circles). This "potentiation"phase lasted 2–5 min after KCl application (results of 6 experiments). Subsequently, the peak diminished (within 1 min) and even disappeared (Fig. 2,B, 7, Fig. 2,C). The phase of "depression"lasted for several tens of minutes (10–40 min).

If during the peak disappearance a membrane-damaging detergent was injected into the area studied (Triton X-100, 20%, 3 μl), resulting in the release of stores of intracellular mediators and their metabolites into the extracellular space, a peak appeared during the first postapplication minute, with an amplitude exceeding severalfold the control level (results not shown).

To test the role of Ca^{2+} in the generation of these phenomena induced by *in vivo* KCl administration into the brain tissue, the solution administered in two experiments (5 μl) contained 0.25 mol.l^{-1} EGTA in addition to 0.5 mol.l^{-1} KCl in 10 mmol.l^{-1} HEPES (pH 7.4). It is obvious from the results illustrated in Fig. 2,*C* (filled circles) and from the recordings in Fig. 2,*D* that EGTA largely suppressed the KCl-induced effects: "potentiation" was reduced and it was of short duration;

the "depression" phase was minimal or absent, even when otherwise KCl alone caused the peak to disappear.

Discussion

Changes of CA.OC induced by K^+ depolarization of isolated synaptosomes from corpus striatum were recorded *in vitro* and in vivo using the technique of voltammetry. *In vitro* experiments were allowed by the employment of a patent protected microthermostatted chamber (Murgaš and Padúch 1989). These studies were prompted by the fact that voltammetry had previously not been employed to take records from extrasynaptosomal fraction; a single report concerning results obtained from *in vitro* experiments on isolated slices of corpus striatum has been available only (Palij et al.1990). An additional reason was an attempt to compare changes associated with stimulus-secretion coupling in vitro and *in vivo* using the same experimental technique the advantages of which had repeatedly been confirmed in experiments with the recording from the brains of waking or anesthesized animals (Gonon et al. 1984; Mermet and Gonon 1986; Rivot et al. 1987; Mermet and Gonon 1988; Milne et al. 1989; May and Wightman 1989; Chien et al. 1990).

A considerable body of evidence has accumulated suggesting that K^+-induced membrane depolarization elicits Ca^{2+} transport across membrane and activates processes involved in the release of mediators from vesicular and non-vesicular storage sites (Nachshen and Blaustein 1980; Akerman and Nicholls 1983; Duncan 1983; Collard et al. 1988; Kauppinen et al. 1988; Turner and Goldin 1989; Nicholls 1989). No CA.OC could be detected in the environment of synaptosomes freshly isolated from corpus striatum (Fig. 1,*a*). No peak appeared even after damaging the synaptosomal membranes with a detergent, suggesting a substantial reduction of metabolic and endogenous transmitter availability of catecholamine substances by the routinely used isolation procedures. Gradual addition of 1–5 $\mu mol.l^{-1}$ DA + 20 $\mu mol.l^{-1}$ pargyline into the incubation medium in the absence of synaptosomes allowed voltammetric calibration of the *in vitro* system. Addition of DA to synaptosomes in incubation medium raised the levels of electroactive substances in these preparations, and the change was voltammetrically detectable (Fig. 1,*b*). Variation of the ionic composition of the incubation medium confirmed the calcium-dependent nature of the mechanisms controlling the concentrations of electroactive substances in the extrasynaptosomal space: raising the concentration of K^+ alone in the EGTA medium had no effect on CA.OC. The reduction of CA.OC observed in the presence of Ca^{2+} ions may be connected with the increased permeability of the plasma membrane of isolated synaptosomes for Ca^{2+} and Na^+ ions (Carvalho et al. 1989; Varečka et al. 1989, Orlický and Varečka 1990) and the secondary elevation of ionic concetration in the intrasynaptosomal compartment and the resulting stimulation of metabolism (Dagani and Erecinska 1987)

and DA uptake (Bigornia et al. 1988). Very likely, these changes were the reason for K^+-induced depolarization of similar synaptosomal preparations not increasing CA.OC. The non-uniform effect of Ca^{2+} ions on K^+- predepolarized synaptosomes (increase but also decrease of CA.OC) may be the result of the reported variability of the transmembrane ionic transport in synaptosomal preparations (Tibbs et al. 1989; Orlický and Varečka 1990). Also, differences in experimental conditions and approaches (time sequence of application and concentration ratios of substances administered) may contribute a deal to this variability. The change in experimental protocol (triggering the processes studied by injecting DA-loaded synaptosomes incubated in calcium-free medium into the depolarizing K^+ environment containing Ca^{2+} ions) provided immediate parallel action of K^+ and Ca^{2+} ions; moreover, the initial phase of K^+-depolarization - induced changes in extrasynaptosomal space could be followed. The observed increase of CA.OC (Fig. 1,*c*) following depolarization was of short duration (approx. 30 s); a maximal value was observed at 90–120 $mmol.l^{-1}$ KCl in the presence of 0.75 $mmol.l^{-1}$ $CaCl_2$, which is in good agreement with the results of *in vitro* classical biochemical methods (Adam-Vizi and Ligeti 1986; Adam-Vizi and Ashley 1987).

In vivo experiments showed that local microinjection of 0.5 $mol.l^{-1}$ KCl in 10 $mmol.l^{-1}$ HEPES (pH 7.4) into the corpus striatum induced marked, multiphase changes in CA.OC. The first phase represented by the growth of the peak amplitude (Fig. 2,*B*, *C*) corresponds to voltammetric observations of electrical stimulation-elicited depolarization in corpus striatum (May and Wightman 1989). As for K^+-induced depolarization, it has been known that microdialysis of corpus striatum with KCl results in an elevation of the concentrations of DA and DA metabolites (as detected by HPLC) in the dialysate (Fairbrother et al. 1990). Our results showed that after K^+-induced depolarization, the increase of CA.OC is followed by a phase of a marked reduction or even disappearance (Fig. 2,*B*, *C*). As the K^+-induced phase of CA.OC increase could be largely blocked by EGTA microinjection (Fig. 2,*C*, *D*) it can be assumed that the mechanisms responsible for that particular phase are Ca^{2+} dependent and that the electroactive substances released are of vesicular origin (Fairbrother et al. 1990). The phase of the marked decrease of the electroactive substance levels in the extracellular space may be the result of a simultaneous action of several mechanisms. One possible explanation is restoration of vesicular stores of mediators based on Ca^{2+}-stimulated metabolism and/or reuptake of the mediators and their metabolites by nerve cells, and clearance by glial elements (Sharp et al. 1986; Gordon et al. 1990). The lacking of the phase of CA.OC depression following EGTA microinjection (Fig. 2,*C*, *D*) confirmed the key role played by Ca^{2+} ions in these processes.

The voltammetric observations presented herein have confirmed the different characteristics of K^+-depolarization induced changes in isolated synaptosomes as compared with those in the microenvironment of intact brain structure. Obviously,

data concerning stimulus-response coupling obtained from *in vitro* experiments, though remaining a valuable source of information, can objectively be interpreted only based on their comparison with results obtained from *in vivo* experiments; for these purposes, voltammetry represents a useful approach.

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