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Transport of an Antihypoxic Drug Stobadine across the Blood-Brain Barrier in Rat Striatum and its Influence on Catecholamine-Oxidative Current: A Voltammetric Study under Normal and Anoxic/Ischaemic Conditions

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Summary

Differential pulse voltammetry with a carbon fibre microelectrode (ME) was used in pentobarbital-anaesthetized rats for monitoring the stobadine current (STB.C) on both sides of the blood-brain barrier (BBB) in the arterial bloodstream (BS) and in the corpus striatum (CS). The STB.C exhibited a distinct peak at a polarization voltage 540±30 mV (n=4). The maximum of STB.C in BS attained 2–3 min after the STB administration (2.8 mg/100 g in 1.0 ml saline solution i.a.) was followed by a rapid decrease to about 20 % within next 3 min. The STB readily passed across the BBB: the STB.C peak appeared in the CS in the 3rd minute and continued to rise up to the 30th min. The administration of STB did not prevent a large increase (1347±326 %, n=3) of the catechol-oxidative current (CA.OC) occurring in the CS between the 4th and 5th minute after cardiac arrest. However, a decrease of ME sensitivity to CA.OC in the presence of STB was observed. This fact leads to the speculation whether a similar "quenching" of dopamine by STB could not participate in the protective effects of STB observed in the brain exposed to hypoxia-reoxygenation.

Key words

Blood-brain-barrier – Stobadine – Catecholamine – Ischaemia – Striatum – Rat

Introduction

Continuous monitoring of the brain concentration of neurotransmitters, metabolites and drugs is important for understanding their action in the CNS. It appeared of interest to investigate the transport of drugs across the blood-brain barrier (BBB) and their influence upon neurotransmitters and metabolites under various pathophysiological conditions.

The merits of the voltammetric technique (high temporal resolution, high spatial resolution and discrete measurement probes) makes it possible to estimate "real time" measurements of the electroactive neurotransmitter content (catecholamines – Adams 1978, Justice 1987) in the extracellular micro-enviroment of selected brain areas. Such observations were made under various pathophysiological conditions of the brain such as an increase of extracellular potassium concentration (Murgaš *et al.* 1991, Pavlásek *et al.* 1992), spreading cortical depression (Pavlásek *et al.* 1993), epileptic activity (Pavlásek *et al.* 1994a) or following an anoxic/ischaemic insult (Murgaš and Pavlásek 1990), and electroconvulsive stimulation (Pavlásek *et al.* 1994b,c).

Voltammetry can be very useful in monitoring the permeation of electroactive drugs through the BBB. We have observed in preliminary experiments that stobadine (STB – Fig. 1A), a novel pyridoindole compound (Beneš and Štolc 1989), gives a distinct redox reaction in the range of voltages applied in *in vivo* voltammetry (Pavlásek *et al.* 1993, unpublished results). It is known that STB, revealing antioxidant (Ondriaš *et al.* 1989, Mišík *et al.* 1991, Horáková *et al.*

1992, 1994, Štefek *et al.* 1992) and free radical scavenging effects (Staško *et al.* 1990, Štefek and Beneš 1991, Steenken *et al.* 1992, Štefek *et al.* 1992, Kagan *et al.* 1993, Horáková *et al.* 1994), is able to protect synaptic transmission in hippocampal slices and in superior cervical ganglia *in vitro* against hypoxia-reoxygenation injury (Štolc 1994, Štolc and Selecký 1995). Besides, the production of conjugated dienes and thiobarbituric acid-reacting substances, the markers of membrane lipid peroxidation induced by severe ischaemia/hypoxia followed by reperfusion reoxygenation, was suppressed in the rat brain under *in vitro* and *in vivo* conditions (Štolc and Horáková 1988, Horáková *et al.* 1990, 1991).

In this study we present data about the dynamics of STB transport across BBB and about its effects upon the catecholamine content in the microenvironment of the brain. Possible mechanisms of the STB protective effects under hypoxia conditions are discussed.

Methods

The experiments were carried out on male Wistar rats with an average body weight of 260 g. Animals were anaesthetized with pentobarbital (Spofa, Prague), 5 % solution in 0.9 % saline, 0.1 ml/100 g b.w., i.p.; about one third of this dose was added after approximately 40 min (duration of the experiment usually did not exceed 120 min).

The animals were fixed in a stereotaxic apparatus and three small openings were drilled in the skull for three voltammetric electrodes:

a) A working (W) glass carbon fibre electrode (Pavlásek *et al.* 1994b,c), treated electrochemically (Mermet and Gonon 1988) was placed in the left corpus striatum with stereotaxic coordinates AP −1.0, L+2.0, V +3.5 to +4.5 (Fifková and Maršala 1960).

b) An auxiliary (AX) electrode (a stainless steel watchmaker's screw) was positioned in the parietal region of the right hemisphere.

c) A reference (R) electrode (Ag/AgCl wire) was inserted into the frontal region of the right hemisphere. The incision in the dura mater was made for the W electrode only; AX and R electrodes were placed epidurally.

Differential pulse voltammetry – DPV (Justice 1987, Pavlásek *et al.* 1994b) was used for recording of electrochemical signals. A polarographic analyzer (PA4, Laboratorní přístroje, Prague) for DPV was connected with a three-electrode system with the following parameters: speed of the linear potential sweep 100 $mV.s^{-1}$, potential limits from −100 mV to +1200 mV, pulse amplitude 50 mV, pulse duration 60 ms (current sampling 20 ms before the start of the pulse and again 20 ms before the end of the pulse), pulse period 0.2 s. The interval between two consecutive voltammetric recordings was 1 min. The voltammetric signal was drawn with an x-y plotter (XY 4106, Laboratorní přístroje, Prague).

Four sets of *in vivo* experiments were performed. In the control series (3 rats), after a period when stabilized recordings had been achieved (10−15 min), voltammetric signals representing a catechol-oxidative current (CA.OC) in the striatum were recorded during the following 30 min.

In the second series (4 rats), after a stabilizing period (10−15 min), five to ten control voltammetric recordings were taken in blood and in the striatum and immediately afterwards stobadine (Fig. 1A) had been administered *via* a cannulated tail artery (2.8 mg/100 g, in 1 ml of a freshly prepared solution with saline, injected within approximately 1.5 min). As far as the minimal differences in body weight (bw, in grams) were concerned, the same initial STB concentration (1.5 mM) in circulating blood was to be attained in each experiment according to the formula used for the calculation of blood volume (Lee and Blaufox 1985): V(ml) = 0.06 bw + 0.77. The voltammetric signals representing CA.OC and STB redox current (STB.C) were recorded in the blood and the corpus striatum during the following 30 min. For the voltammetric recordings in the circulating blood, the left femoral artery and the right femoral vein were cannulated and a special closed flow chamber was used (Fig. 1B).

In the third, untreated series (3 rats), after period in which stabilized CA.OC recordings in the striatum were achieved (10−15 min), five to ten control recordings were obtained in each animal and immediately afterwards cardiac arrest (verified by palpation) which had been induced by an intracardial air injection (0.5 ml). The CA.OC was registered in the striatum for another 30 min.

In the fourth series (4 rats), cardiac arrest was preceded by an STB injection (2.8 mg/100 g, in 1 ml of a freshly prepared saline solution, injected into the tail artery during 1.5 min). Voltammetric signals representing CA.OC in the striatum were recorded for further 30 min after cardiac arrest.

Calibrations of the STB.C and dopamine redox current in saline solutions with different STB and dopamine concentrations were performed *in vitro*.

The quantification of the electrochemical signals was obtained by measuring the amplitude of the peaks representing the redox current.

The Student's t-test was used to evaluate the results (arithmetic means and standard deviations are shown).

Compounds used:

Stobadine [(-cis-2,8-dimethyl-2,3,4a,5,9b-hexahydro-1H-pyrido(3,3b) indole .2HCl] was supplied by the courtesy of Professor L. Beneš, Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic. Dopamine was from Sigma, USA. All other chemicals used were of analytical grade.

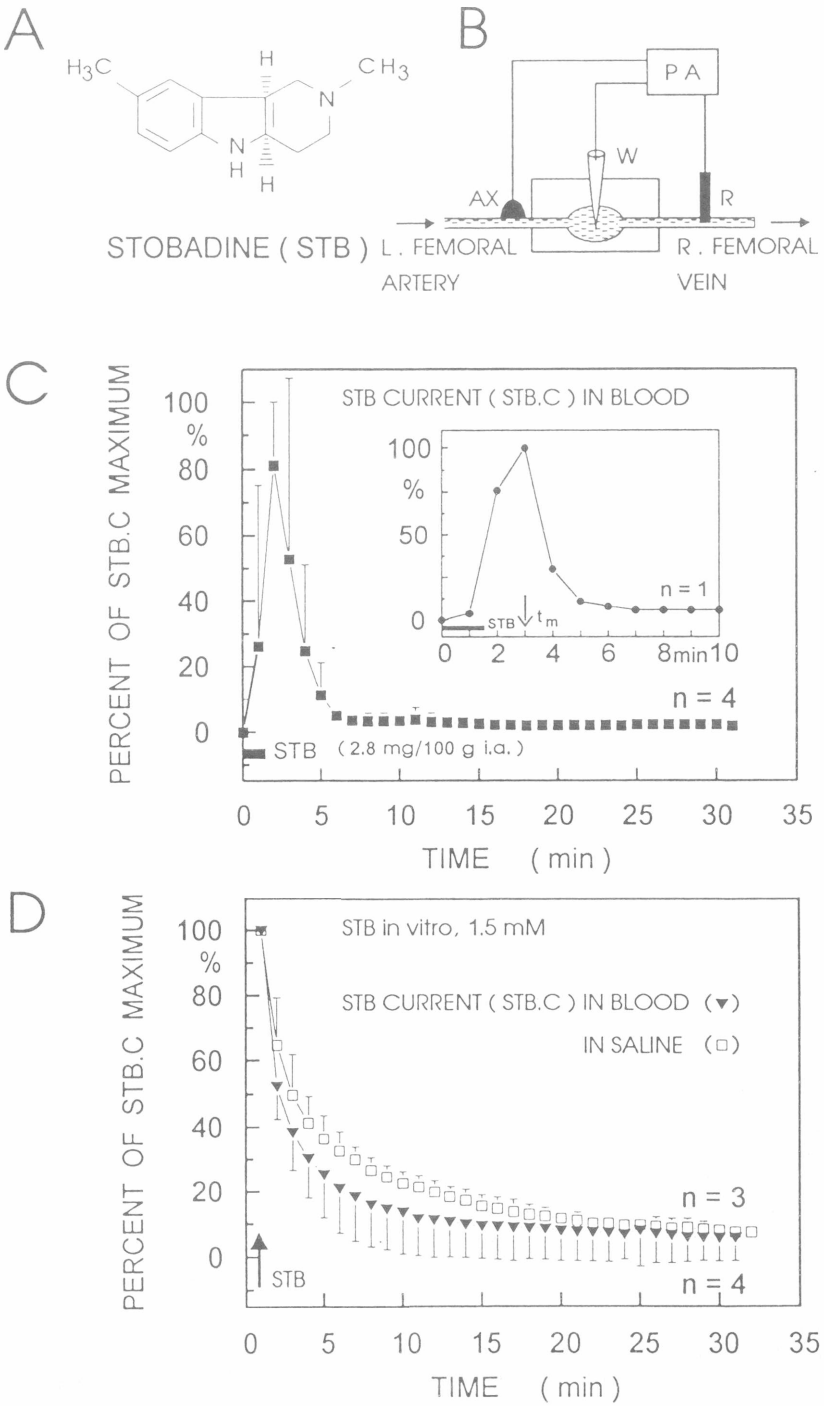

Fig. 1

Detection of stobadine with the voltammetric technique in the blood and in saline solution.

A. Structure of the stobadine (STB) compound.

B. Schematic sketch of a closed flow chamber used for monitoring STB in circulating blood. PA – polarographic analyzer working in the differential pulse voltammetry mode with a three-electrode system: AX – auxiliary, W – working, R – reference.

C. Time-course of STB redox current (STB.C) changes in the circulating blood. STB administration into tail artery (2.8 mg/100 g) indicated by horizontal black bar. Maximal STB.C was set as 100 % in each experiment (n = 4). The inset documents result from one experiment; downward pointing arrow denotes time (t_m) in which STB.C maximum was attained. Vertical bars in Figs. 1, 4, 5 and 6 represent S.D.

D. Time-course of STB.C changes recorded in vitro. Black symbols: 1.5 mM STB in 1 ml blood samples (n = 4). Open symbols: 1.5 mM STB in saline solution (1 ml, n = 3). The initial (maximal) STB. C recorded shortly after STB dissolved in 0.1 ml saline solution was added (upward pointing arrow) was set in each case as 100 %.

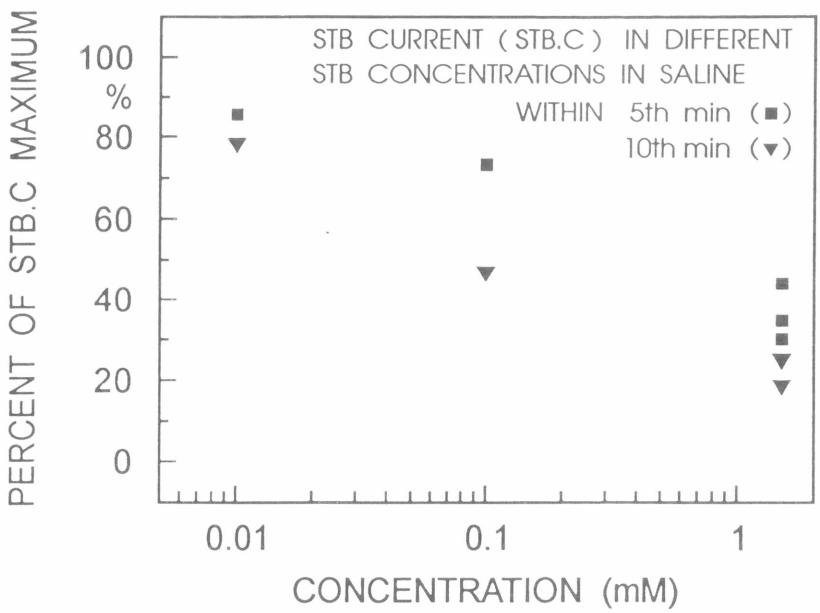

Fig. 2
The influence of the stobadine (STB) concentration and time of measuring with the voltammetric technique on the stobadine redox current (STB.C). The results obtained in saline solution with 0.01 mM, 0.1 mM and 1.5 mM STB in the 5th min (squares) and in the 10th min (triangles) are presented. Each symbol represents one calibration. The initial (maximal) STB.C was set as 100 %.

Results

1. Stobadine redox current in the circulating blood

Stobadine (Fig. 1A) revealed a distinct peak at a polarization voltage 540±30 mV (n=4) by polarographic measurements in circulating arterial blood (Fig. 1B). The time-course of STB.C was characterized by a rapid increase: maximum (26±14 nA, n=4) was attained 2–3 min after starting STB administration into the tail artery and an equally fast decrease to a level lower than 10 % of the STB. C maximum about 6 min after the onset of the STB injection (Fig. 1C). The fast decline of STB.C can be the result of various mechanisms such as drug transport into the blood cells, drug-protein interaction in the plasma, chemical interaction with other molecules, distribution into the extravascular compartments in the body, drug metabolism, drug elimination and last, but not least, the result of diminishing sensitivity of the W electrode. We tested these possibilities in *in vitro* experiments as it is well known that STB in aqueous solutions is stable at room temperature for several days.

2. Stobadine redox current in vitro

2.1. Calibrations in the blood

STB was added to a defined volume (1.0 ml) of the arterial blood sample in an amount to achieve 1.5 mM concentration (identical to that supposedly attained in the circulating blood after the STB i.a. injection). The maximum of the STB peak (11±9 nA, n=4) in the blood samples was clearly identifiable at a polarization voltage 540±30 mV (n=4). A rapid decline of the STB.C was observed (Fig. 1D, black symbols). The STB.C curve approximated the equation $y = A * x^B$ with the parameters: A = 91.3, B = −0.82, and regression coefficient r=0.997. The STB.C decrease in blood sample *in vitro* was not appreciably different from the STB.C decrease in the circulating blood. This fact provided evidence that STB distribution into the extravascular compartments and/or drug elimination were not the decisive mechanisms responsible for STB.C reduction in circulating blood.

2.2. Calibrations in saline

STB was added to a defined volume (1.0 ml) of freshly prepared saline in an amount to achieve 1.5 mM concentration. The voltage of the maximum STB peak (35±8 nA, n=3) was at 595±20 mV (n=4) (Fig. 3B,b). A rapid decline of STB.C was observed (Fig. 1D, open symbols). The STB.C curve approximated the equation $y = A * x^B$ with the parameters: A = 123.6, B = −0.78, and regression coefficient r=0.992. The STB.C decrease in saline did not differ substantially from STB.C lowering in blood samples *in vitro*. This fact proved that drug transport into blood cells, drug-protein interaction in the plasma, chemical interaction with other molecules, drug metabolism and protein poisoning of the W electrode were not crucial mechanisms responsible for the STB.C reduction in circulating blood.

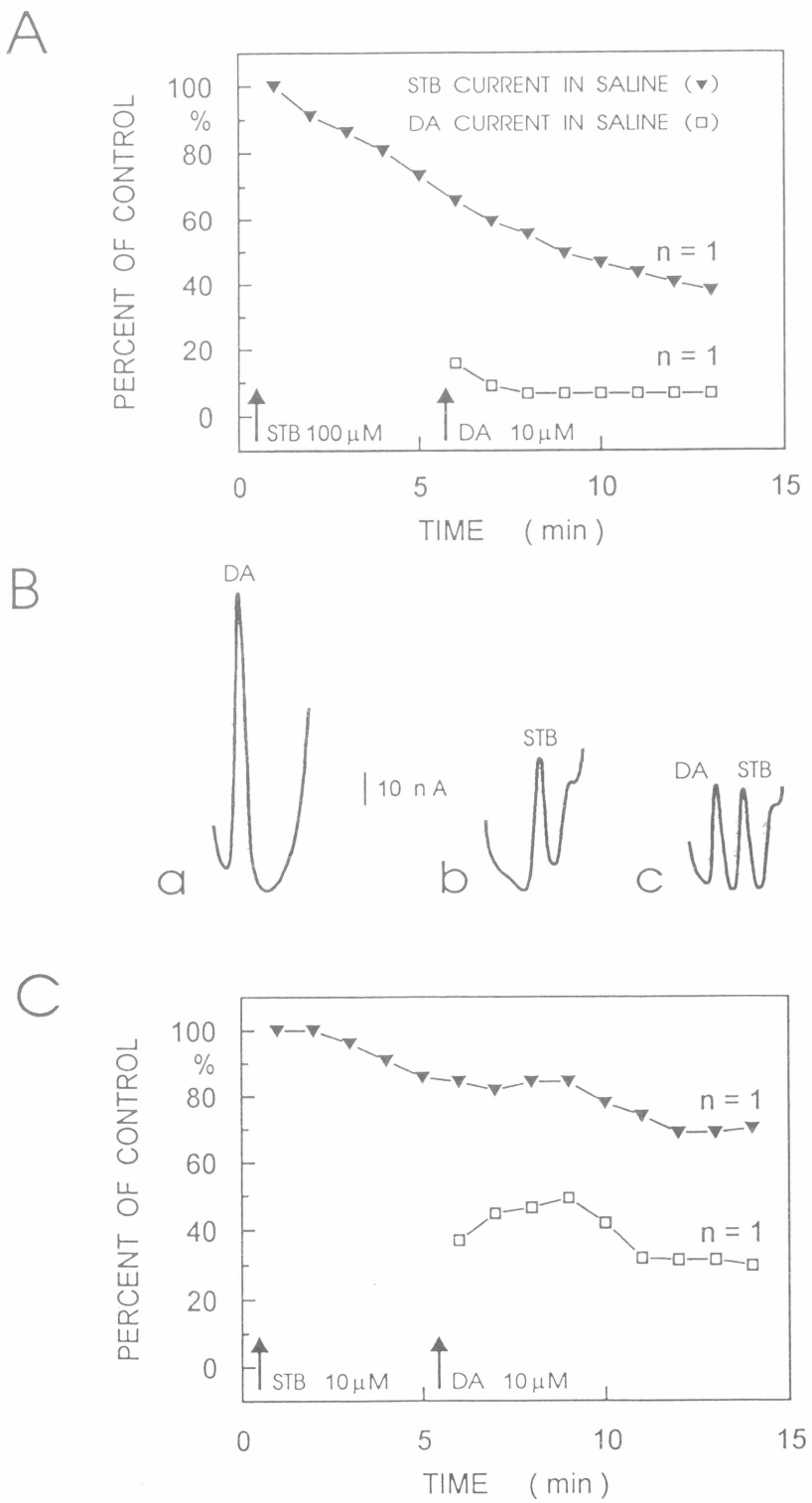

Fig. 3

The stobadine redox current (STB.C) and dopamine redox current (DA.C) in saline solution as determined with the voltammetric technique.

A. The result obtained in saline solution with 0.1 mM STB to which dopamine (DA) was added (0.01 mM) in the 6th min of the recording (upward pointing arrow). The initial (maximal) STB.C (triangles) was set as 100 %. Calibration in saline solution containing 0.01 mM DA represented control (100 %) for DA.C (squares).

B. The DA.C and the STB.C in saline solution. a – DA.C peak in saline with 0.01 mM DA. b – STB.C peak (initial, maximal amplitude) in saline with 0.01 mM STB. c – DA.C and STB.C in saline: illustrated record was taken in the 10th min after the registration started in 0.01 mM STB solution and 5 min after DA was added (0.01 mM).

C. The same in vitro measurement as in A, in a solution containing 0.01 mM STB.

The STB.C drop in saline solutions depends on two factors. The higher the STB concentration and/or the longer the measuring time, the more evident is this effect (Fig. 2). At the end of the 10 min measuring period in 10 μM STB solution, STB.C decreased to 78 % of its maximal (i.e. initial) value; the difference in comparison with the 5 min period (86 %) was small. In a 100 μM STB solution, the STB.C decreased to 73 % (47 %) of the maximum during the 5 min (10 min) measuring interval. The STB.C in 1.5 mM STB solution reached values ranging from 44 % to 35 % (from 25 % to 18 %) of the maximum at the end of the 5 min (10 min) measuring interval. Decline of the W electrode sensitivity in relation to STB concentration (c) in the 10 min period approximates the line equation $y = A + B * \ln c$ with the parameters: A = 25.1, B = −11, regression coefficient r = 0.992. It is evident that STB.C is notably reduced by STB concentrations close to and above 100 μM.

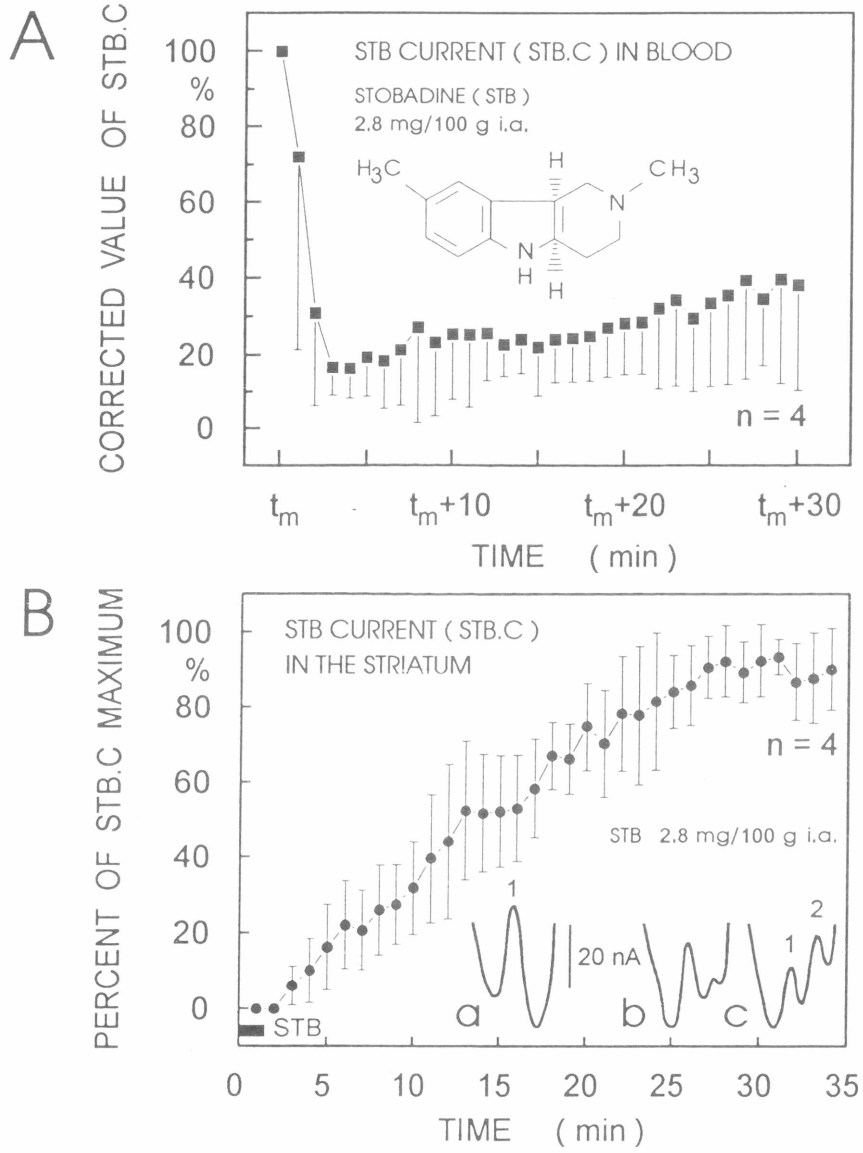

Fig. 4

The time-course of the stobadine redox current (STB.C) changes in the circulating blood and in the corpus striatum as observed with voltammetry.

A. Corrected values of the STB.C (see the text) measured in the circulating arterial blood (see Fig. 1 B) after STB administration into the tail artery (2.8 mg/100 g). Maximum of the STB.C observed at time t_m was set as 100 % in each experiment (n = 4). The inset represents the STB compound.

B. Time-course of the STB.C changes in the corpus striatum after STB administration (horizontal black bar) into tail artery (2.8 mg/100 g). Maximal STB.C was set as 100 % in each experiment (n = 4). The inset voltammetric records (a – c) document catechol-oxidative current (peak 1) and STB.C (peak 2). a – record taken before STB injection, b, c – records in the 5th and 20th min after the start of STB injection.

Since the STB assay could be influenced by STB interaction with other molecules such as catecholamine derivates occurring in relatively high amounts, both in the blood and in the corpus striatum, further *in vitro* measurements calibrating STB.C were performed in the presence of dopamine (DA) in a saline solution (Fig. 3). The lower voltage 275±25 mV (n=4) of the DA current (DA.C) maximum (Fig. 3B,a) enabled its distinct separation from the STB.C peak (Fig. 3B,c). There was no change in the trend of the STB.C decrease after DA (10 μM) had been added to the STB solution (10 μM – Fig. 3C, 100 μM – Fig. 3A). On the other hand, we observed a marked depression of the DA.C in the presence of STB (Fig. 3B,c) in comparison with the control (10 μM DA in saline solution) – Fig. 3B,a. This depression was more pronounced in the solution with a higher STB concentration: after 10 min, the DA.C was only about 10 % of the control in a solution with 100 μM STB (Fig. 3A), while it was approximately 40 % in a solution with 10 μM STB (Fig. 3C).

3. Dynamics of the stobadine redox current changes on both sides of the blood-brain barrier: blood and corpus striatum

The calibrations *in vitro* clearly demonstrated a marked decrease of the W electrode sensitivity to STB (STB.C lowering) in an environment with STB concentrations approaching 100 μM and above. Therefore, the STB.C values measured in the circulating blood (Fig. 1C) with a high initial STB concentration (about 1.5 mM) had to be corrected. The data correction was made as follows: the attenuation coefficients of the W electrode sensitivity for 1.5 mM STB in the saline were determined at each minute of the recording period. The STB.C values obtained in circulating blood were multiplied by these coefficients in the corresponding minutes of the recording. The corrected STB.C values are illustrated in Fig. 4A. The maximum of the STB peak signal in the arterial blood achieved 2.3±1 min (n=4) after the onset of STB administration (time t_m) into the tail artery was considered to be 100 %. There was rapid decrease to about 20 % of its maximum within the next three minutes and no further significant changes were observed till the end of the measurement (t_m + 30 min).

In the striatum maximum of the STB.C (15.4±6.7 nA) occurring at the voltage 540±30 mV (n=4) was distinctly separable from the first peak attaining its maximum at a lower polarization voltage 360±30 mV (n=4) – Fig. 4B,a–c. It is known that in the DPV mode the peak formed at the voltage near 400 mV represents CA.OC (Pavlásek et al. 1994b).

The time course of the STB peak changes in the striatum is illustrated in Fig. 4B. In this graph, the maximum of the STB.C in each experiment was taken as 100 %. The STB signal appeared in the third minute after the STB injection had started and rose continuously up to the 30th min.

The DPV records from the striatum (insets a-c in Fig. 4B) document the the progressive diminution of CA.OC (peak 1) after STB injection, while the STB.C (peak 2) was still rising. The time-course of the CA.OC changes in the striatum after STB administration is shown in Fig. 5. The CA.OC lowering started in the third minute after the beginning of the STB injection and continued till the 20th minute when CA.OC became stabilized at a level of about 30 % of the control.

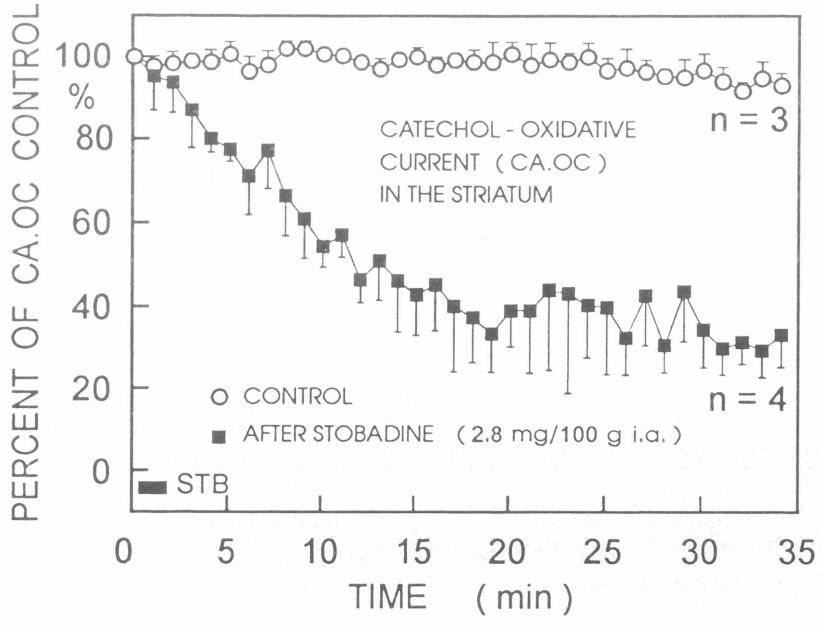

Fig. 5
The influence of stobadine (STB) administration (horizontal black bar) into tail artery (2.8 mg/100 g) on the catechol-oxidative current (CA.OC) in the striatum as assessed by voltammetry. Open symbols – CA.OC in control experiments (n=3): the mean of the last 5 records preceding the measuring period (before time 0 min) served as control (100 %) in each experiment. Black symbols – CA.OC in experiments with STB administration (n=4); the mean of the last 5 records taken prior to STB injection served as control (100 %) in each experiment.

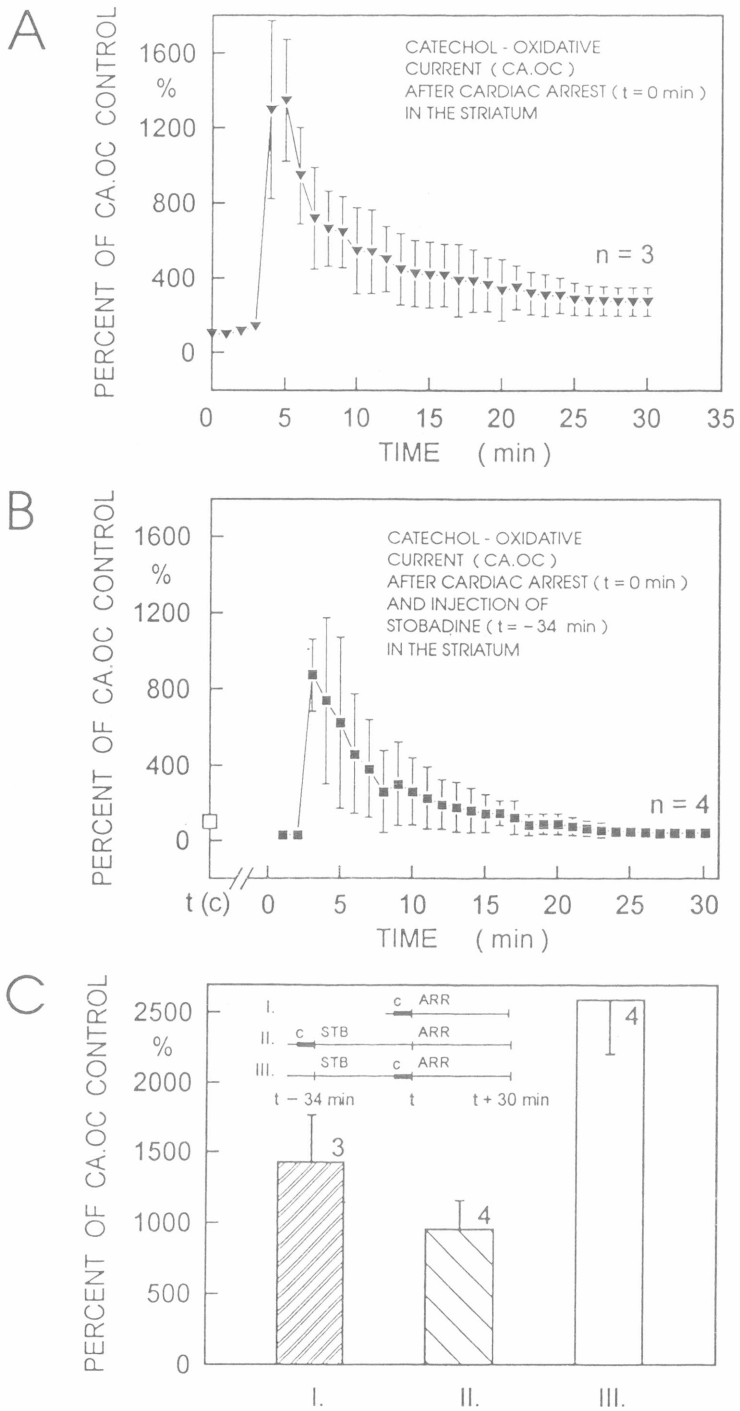

Fig. 6

Changes of the catecholamine-oxidative current (CA.OC) in the corpus striatum after cardiac arrest: the influence of stobadine (STB) as determined with voltammetry.

A. Time-course of the CA.OC changes after cardiac arrest onset at time t = 0 min. The mean of the last 5 records preceding measuring period (before time 0 min) served as control (100 %) in each experiment (n = 3). See also column I and inset I in part C.

B. Time-course of the CA.OC changes after cardiac arrest onset at time t = 0 min and STB injection into tail artery (2.8 mg/100 g) 34 min earlier (t = −34 min). The mean of the last 5 records taken at time t (c) before STB injection (before time −34 min) served as control (100 %, open square at the ordinate) in each experiment (n = 4). See also column II and inset II in part C.

C. The maximal CA.OC values observed after cardiac arrest (column I) and after cardiac arrest (ARR) preceded by STB injection (columns II and III). The time in which control (c) recordings were taken was different in the case of the results represented by columns II and III (compare the insets II and III). The digits near (in) the columns represent the number of experiments.

4. Changes of the catechol-oxidative current in the striatum during anoxic/ischaemic insult: the influence of stobadine

There was a large increase of CA.OC in the striatum during anoxic/ischaemic insult (Fig. 6A). The mean of the CA.OC values reached almost 1400 % of the control (1347±326 %, n=3) between 4th and 5th minute (t_m) after cardiac arrest. An exponential drop of CA.OC following t_m continued till the registration was stopped (30th min); at the end of this period CA.OC was still significantly higher in comparison with the control.

In experiments with STB injection (2.8 mg/100 g i.a) applied prior to cardiac arrest (Fig. 6B,C), the mean of the CA.OC values in the striatum attained almost 900 % of the control (870±190 %, n=4) between 3rd and 4th minute (t_m) after cardiac arrest. The CA.OC decrease following t_m lasted till the 25th minute of the registration period (Fig. 6B).

The lower value of the mean of the maximal CA.OC increase in animals with STB administration preceding cardiac arrest (compare Fig. 6B with Fig. 6A) could be the outcome either of mechanisms affecting metabolic turnover of catecholamines and their release or of an altered electrochemical reaction induced by STB at the W electrode surface (see section 2.2). Though the observed difference was insignificant, we tried to analyze which of the mentioned assumptions was correct. For this purpose, two controls were taken in each experiment with STB application: the first one before STB application and cardiac arrest and the second one after STB injection and before cardiac arrest. Their time sequence in the course of an experiment is schematically illustrated in insets II and III in Fig. 6C. The mean of maximal CA.OC elevation expressed in percentage of the control taken prior to STB injection (Fig. 6B, Fig. 6C column II) was significantly lower in comparison with the CA.OC rise expressed in percent of the control taken after STB administration (Fig. 6C, column III). These facts can be explained as follows: the control value of CA.OC taken after STB administration is lower than that obtained before STB injection because of sensitivity changes of the W electrode; therefore, the alteration of CA.OC expressed in percent of the lower control is greater and *vice versa*. These results indicated that STB probably did not prevent catecholamine release into the extracellular space induced by the anoxic/ischaemic insult.

Discussion

The free fraction of the drug in the blood is one of the important determinants of its diffusion across the BBB. Voltammetry enables the monitoring of levels of free (unbound) fraction of compounds on both sides of the BBB – in the plasma and in the extracellular space of the selected brain areas. The importance of this information follows from the fact that only the free fraction of the drug present in the microenvironment of the brain is available for transport to the receptor compartment. Other techniques frequently used in pharmacokinetic studies such as the detection of radioactively labelled compounds by extraction procedures combined with spectrophotometric analyses usually estimate the total tissue content of a compound regardless of whether it is in the free or bound form.

In a pharmacokinetic analysis it was shown (Kállay *et al.* 1990) that plasma levels of ^{3}H-labelled STB following i.v. administration decayed exponentially from the maximal value with a terminal elimination half-life of 85.6 min.

The present analysis showed that voltammetry can detect STB with a reasonable selectivity. There was a rapid decay of the free fraction of the STB content in the arterial blood following i.a. administration from the initial peak to a relatively steady value (about 20 % of its maximum) which was observed for 30 min. This might indicate a dynamic equilibrium between outflow and inflow of STB free form from the plasma compartment to other compartments and *vice versa*.

Kállay *et al.* (1990) demonstrated that, following i.v. administration, STB passes through the BBB, achieving a peak concentration in the brain within several minutes. The total STB brain content decreases exponentially in the course of the next 5 hours to a very low value. The above mentioned authors reported a high brain tissue/plasma ratio (approximately 10:1) which is in accord with data concerning STB lipophilicity (apparent partition coefficient measured in octanol/phosphate buffer at pH 7.32 and 25 °C, log P' = 0.5315) (Beneš *et al.* 1985). Because of its high lipophilicity, STB is obviously extensively bound to brain matrix components and hence only a small fraction of its total content might be in the extracellular fluid of the brain. Our study demonstrated that STB levels are increased in the extracellular microenvironment of the brain 30 min after STB injection. The slow elevation of the STB free fraction in the aqueous medium might reflect gradual saturation of the lipophilic brain matrix components acting as a sink for the extracellular compartment. One has to be very careful in assessing the concentration of the drug in the tissue on the basis of *in vitro* calibration of the voltammetric signals. It is well known that the speed of an electrochemical reaction at the W electrode surface in the brain can be substantially different in comparison with *in vitro* conditions (Justice 1987). In studies dealing with a lipophilic drug (acetaminophen, log P' = 0.25) and using the technique of intracerebral microdialysis for estimation of its concentration in the extracellular fluid, BBB

transport based on the brain/plasma ratio was 18 % (de Lange 1993). In our experiments, the values of the STB content in the arterial blood, after correction for sensitivity decay of the W electrode, were about 20 % (about 300 μM) of their initial (maximal) values (approximately 1.5 mM). Using the above mentioned brain/plasma ratio (de Lange 1993), we obtained a rough estimation of the maximal STB free fraction concentration in the brain which was in the range of several tens of μM. At such STB concentrations, the sensitivity of the W electrode is only moderately decreased (Fig. 2, Fig. 3C) and one can therefore suppose that the information about the concentration-time profile of STB in the corpus striatum, presented in Fig. 4B, need not be distorted too much.

Cerebral anoxia and ischaemia of different severity lower cellular energy production (Lowry *et al.* 1964, Siesjö 1981) and reduce the efficacy of membrane ionic pumps (Hansen 1985). The consequent depolarizing shift in transmembrane ionic balance results in an increase of $[K^+]_e$, and a decrease of $[Na^+]_e$, and $[Ca^{2+}]_e$, (Silver and Erecinska 1990). Approximately 2.5 min after the onset of ischaemia, the baseline membrane potential of the spiny neurones in the rat neostriatum quickly becomes depolarized towards 20 mV and remains at this level during ischaemia (Xu 1995). Membrane depolarization is accompanied by massive leakage of neurotransmitters into the extracellular space (Benveniste *et al.* 1984, Obrenovitch *et al.* 1990). Dopamine present in high concentrations in the extracellular space of the ischaemic corpus striatum (Richards *et al.* 1993) might be subject to autooxidation. During this reaction, products exhibiting properties of free radicals might be generated (Singal *et al.*, 1983, Olanow 1993). These free radicals were suggested to have toxic effects which seem to be one of the most important factors in anoxic/ischaemic damage (Demopoulos *et al.* 1982, Watson *et al.* 1984, Braughler and Hall 1989, Hall and Braughler 1989, Halliwell and Gutteridge 1992).

As has been shown in our study, STB probably does not prevent massive catecholamine release into the extracellular space of the corpus striatum induced by the anoxic/ischaemic insult (Fig. 6C). The measurements carried out *in vitro* proved that the sensitivity of the carbon W electrode to DA was diminished in the presence of STB (Fig. 4, Fig. 5). A similar effect was observed even if W electrodes were just immersed into an STB solution without application of the linear potential sweep onto them (not documented). The nature of these physicochemical processes is not clear. At the moment one can just speculate whether such "quenching" of DA by STB could participate in the protective effects of STB observed in the brain tissue exposed to hypoxia-reoxygenation. This idea, however, should be verified experimentally.

Acknowledgments

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