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Voltammetrically Determined Differences in Changes Evoked by KCl Microinjections on Catecholamine Levels in the Reticular Formation and Corpus Striatum of the Rat

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Summary

Using a microelectrode with carbon filaments and the voltammetric technique, changes evoked in the catechol oxidation current (CA.OC) and multiple unit activity (MUA) by microinjection of 3-5 μl 0.5 mol.l^{-1} KCl were studied in the reticular formation (RF) of the medulla oblongata of anaesthetized rats; the effect of KCl stimulation of the RF and corpus striatum (S) on the CA.OC in these structures was compared. The microinjection of KCl in the vicinity of the working electrode in the RF caused depression of MUA which began 2-3 s after administration, persisted for up to 6 min after and then diminished, reaching control values within 9 min. The voltammetric signal was first recorded in the 1st min after microinjection, when there was an evident decrease in the CA.OC value (59 % of the control value); this effect reached its maximum 7 min after administration (a mean drop to 23 % of the control), while at the end of the experiment (i.e. after 24 min) CA.OC values had risen to 45-80 % of the control value. The response in the S had a biphasic character, however. Immediately after the microinjection (1st min), the mean CA.OC value rose to 626 % of the control, while in the second phase (3-10 min) it was seen to fall below the control values (means 21-63 % of the control). The differences in the changes evoked by K^+ depolarization in the concentration of catecholamines in the RF and S microenvironment are discussed from the aspect of the existence of different pools of the transmitter and other regional differences. The possibility of a relationship between the above phenomena and the spreading depression wave is also considered.

Key words

K^+ depolarization – Catecholamines – Reticular formation – Striatum – Voltammetry – Rat

Introduction

Changes in the microenvironment in the extracellular space of the central nervous system (CNS) have the nature of chemical stimuli influencing the activity of CNS cells. It is thus obvious that an information channel formed in the CNS by anatomically preformed interneuronal connections is associated functionally with another information channel – i.e. with the extracellular microenvironment of the CNS (Nicholson 1979).

An increase in the extracellular potassium concentration depolarizes the CNS cell membranes and leads to a K^+-stimulated release of transmitters (Paulsen and Fonnum 1989, Fairbrother *et al.* 1990).

Among organic molecules, catecholamines, which act as transmitters and neuromodulators, have an important signalizing function in the microenvironment of the CNS (Krnjevic 1984). Catecholaminergic neurones in the CNS replenish the catecholamine level in the microenvironment partly through output in the region of synapses (with subsequent diffusion into the extracellular space) and partly by non-synaptic, non-quantum release outside the axonal ending zones (Cheramy *et al.* 1981, Tauc 1982). Catecholamine molecules in the CNS microenvironment influence the extrasynaptic membrane receptors of the neurones. By means of these mechanisms – as shown by their ionophoretic administration by way of a microelectrode – catecholamines influence the activity of CNS neurones (Hösli *et al.* 1971, Armstrong-James and Fox 1983, Krnjevic 1984, Hricovini and Pavlásek 1985).

Since catecholamines and their metabolites are by nature electroactive, their level in the extracellular space of the CNS can be monitored *in vivo* by the voltammetric technique (Adams 1978), using a microelectrode with a carbon filament (Gonon

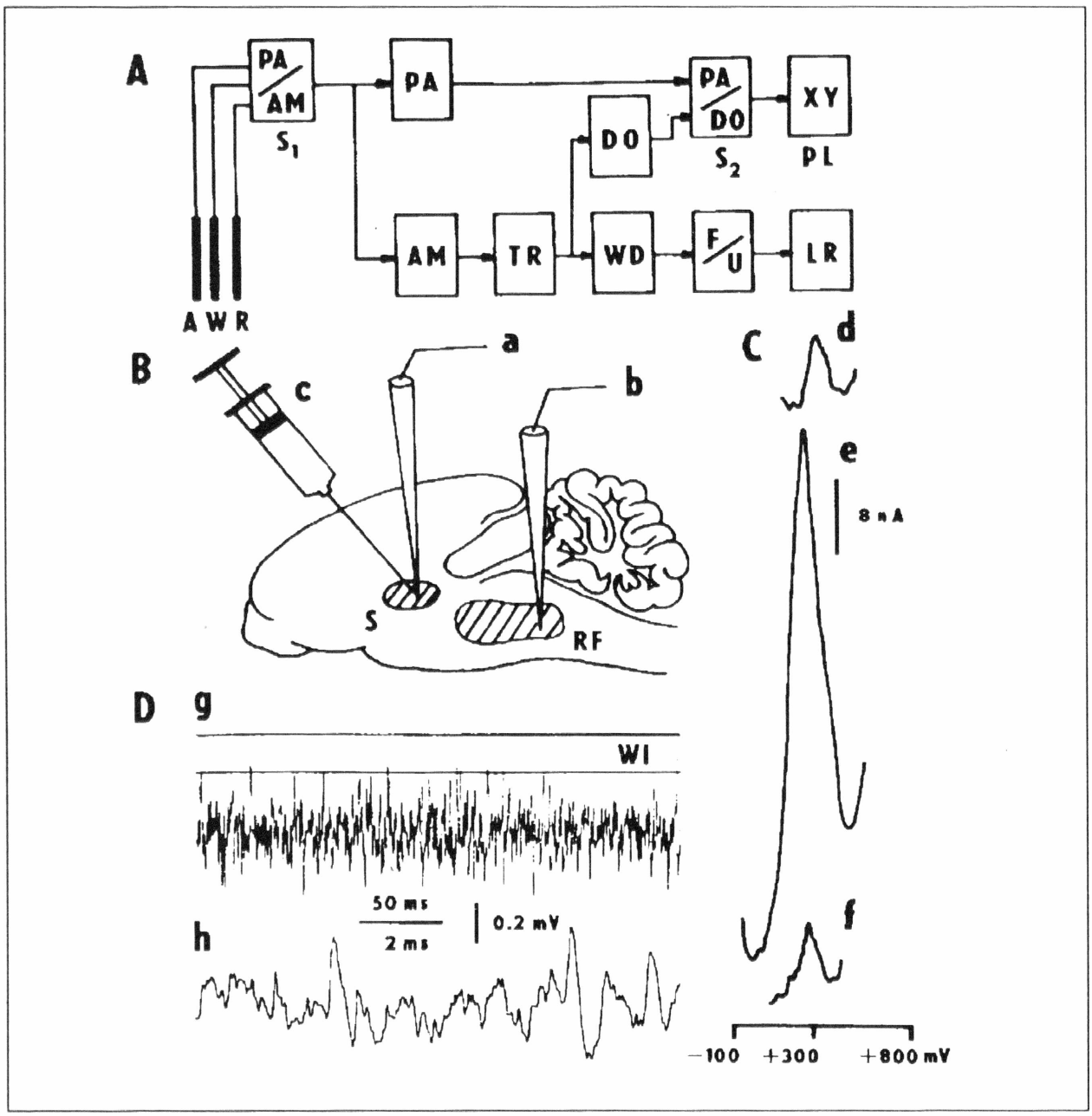

Fig. 1

Schema of the experiment; recording of the voltammetric signal and neuronal activity. A. Block diagram of connection of the apparatus for recording the voltammetric signal and neuronal activity. A – auxiliary electrode, W – working electrode, R – reference electrode, PA – polarographic analyser, AM – amplifier, TR – tape recorder, DO – memory oscilloscope with digital output, WD – window discriminator, PL – x-y plotter, LR – line recorder, F/U – frequency-voltage converter, S1 and S2 – switches. B. Scheme of the sagittally sectioned rat brain. Localization of the recording electrodes and of the micropipette for injecting KCl. The working electrodes (a,b) were localized in the corpus striatum (S) and the reticular formation – RF (hatched). KCl (3-5 μl 0.5 mol.l^{-1}) was administered by micropipette (c) in the immediate vicinity of a and b. C. Effect of the microinjection of noradrenaline (NA) close to the tip of the working electrode in the RF on the catechol oxidation current (CA.OC) in the volammetric recording. d – control, e – recording 7 min and f – 20 min after administering 5 ll 33 lmol.l^{-1} NA (see text). The vertical line (8 nA) calibrates the CA.OC and the horizontal line the polarization voltage on the working electrode (-100 mV to +800 mV). D. The working electrode (b) in the RF was also used for the extracellular recording of spontaneous neuronal unit activity selected on the basis of amplitude differences by an electronic window (WI). Time calibration 50 ms (g) and 2 ms (h), voltage calibration 0.2 mV (g,h).

et al. 1984). As shown by voltammetric observations, changes evoked in catecholamine levels by the action of electric or natural stimuli on the CNS of an experimental animal differ in various CNS structures; this may be determined by the biological significance of the stimuli for the organism and by a priority effect on given CNS pathways (May an Wightam 1989, Bertolucci-D'Angio *et al.* 1990). In concrete parts of the CNS, the structural organization and dynamics of metabolic processes associated with the synthesis of catecholamines and the mechanism of their release, degradation and re-transport back into the CNS cells may also be a co-determinant factor. A comparison of the dynamics of changes in catecholamine levels in individual structures of the CNS, using direct application of the same stimulus into the region under investigation, could help to elucidate the role of these factors.

In the study presented below, the authors compared observations of the dynamics of K^+-evoked changes in the level of catecholamine type substances in the extracellular space of the reticular formation (RF) and corpus striatum (S).

Material and Methods

The experiments were carried out on rats (Wistar strain) with a mean body mass of 250 g, which were anaesthetized with pentobarbital (Pentobarbital Spofa, 5 % solution, 5 mg/100 g i.p.) and then fixed in a stereotaxic apparatus. Apertures for the working electrode, reference electrode (Ag/AgCl) and auxiliary electrode were drilled in the skull and the dura mater was opened in the hole for the working electrode (Fig. 1 A,B).

The working electrode – a glass micropipette with 5-8 carbon filaments approximately 7 μm thick – had a tip diameter of 100-150 μm (the exposed carbon filaments were about 200 μm in length). Before starting the measurement, the surface of the working electrode was treated electrochemically (Mermet and Gonon 1988). Using a micromanipulator, a glass micropipette (tip diameter $\leq$ 100 μm) was placed in the immediate vicinity ($\leq$ 1.0 mm) of the working electrode for pressure administration (Pavlásek and Dekan 1986) of 0.5 mol.l^{-1} KCl in a dose of 3-5 μl.

The stereotaxic coordinates of the corpus striatum (S) and the gigantocellular nucleus of the reticular formation (RF) in the medulla oblongata were determined according to the atlas of Fifková and Maršala (1960); S: 1 mm rostrally to the bregma, 2 mm laterally from the midline and 3.5-4.5 mm vertically, below the surface of the brain; RF: 9 mm caudally to the bregma, 1 mm laterally from the midline and 7-8 mm vertically, below the surface of the brain.

When stabilized, the voltammetric signal was measured at regular intervals. The polarographic analyser (PA 4, Laboratory Equipment, Prague) worked with a three-electrode system (Fig. 1 A) in a differential pulse voltammetry (DPV) mode with the following parameters: rate of the increase in the polarization voltage 100 mV.s^{-1}, pulse amplitude 100 mV, pulse duration 60 ms, pulse period 0.2 s; the voltage supplied to the working electrode ranged from -150 mV (in some cases from -100 mV) to +800 mV. The voltammetric signal was drawn with an x-y plotter (XY 4106, Laboratory Equipment, Prague).

In the time periods between recordings of the voltammetric signal, the working electrode in the RF was used for continuous recording of multiple neuronal activity which was recorded on a tape-recorder and observed simultaneously on a Gould 4121 oscilloscope; the contents of the oscilloscope's memory were drawn on an x-y plotter (XY 4106). The frequency of spike potentials selected by a window discriminator (WD) was transformed by means of a F/U converter to a continuous voltage signal, which was recorded with a TZ 4200 line recorder (Fig. 1 A).

Results

Characteristics of recordings in the corpus striatum and reticular formation

Two clearly identifiable peaks were found in the curves from the RF (Fig. 2 A,b) and S (Fig. 3 C,a). In the RF, the first one attained the maximum at +97 ± 26 mV on the working electrode and the second at +390 ± 33 mV (the arithmetical means ± S.D. in 9 experiments). The values in the S were 115 ± 31 mV for the first maximum and +374 ± 26 mV for the second (means of 10 experiments). It is known from the literature that, in the DPV mode, the peak formed at the lower voltage corresponds to the ascorbic acid, while the other represents the current from catecholamines and their metabolites, i.e. the CA.OC (Lane *et al.* 1976). The CA.OC was verified by injecting noradrenaline (NA) close to the working electrode (Fig. 1 C,d-f). The administration of 5 μl freshly prepared 33 μmol.l^{-1} NA (bitartrate salt in physiological saline, total dose 0.08 μg) raised the CA.OC to 1140 % of the control value; a progressive return (710 % 7 min after microinjection – Fig. 1 C,e) to the initial value was observed 15 min and more after the administration of NA (Fig. 1 C,f).

The size of the CA.OC depended on the length of the regular time interval between measurements (Tab. 1). At each of the given intervals a series of six measurements was carried out (the mean of the 2nd to 6th measurement was compared with the first measurement in the relevant interval). If the interval between measurements was shorter than 5 min, the CA.OC decreased. The decrease in the RF (to 84 % with a 3 min interval and to 27 % with a 1 min

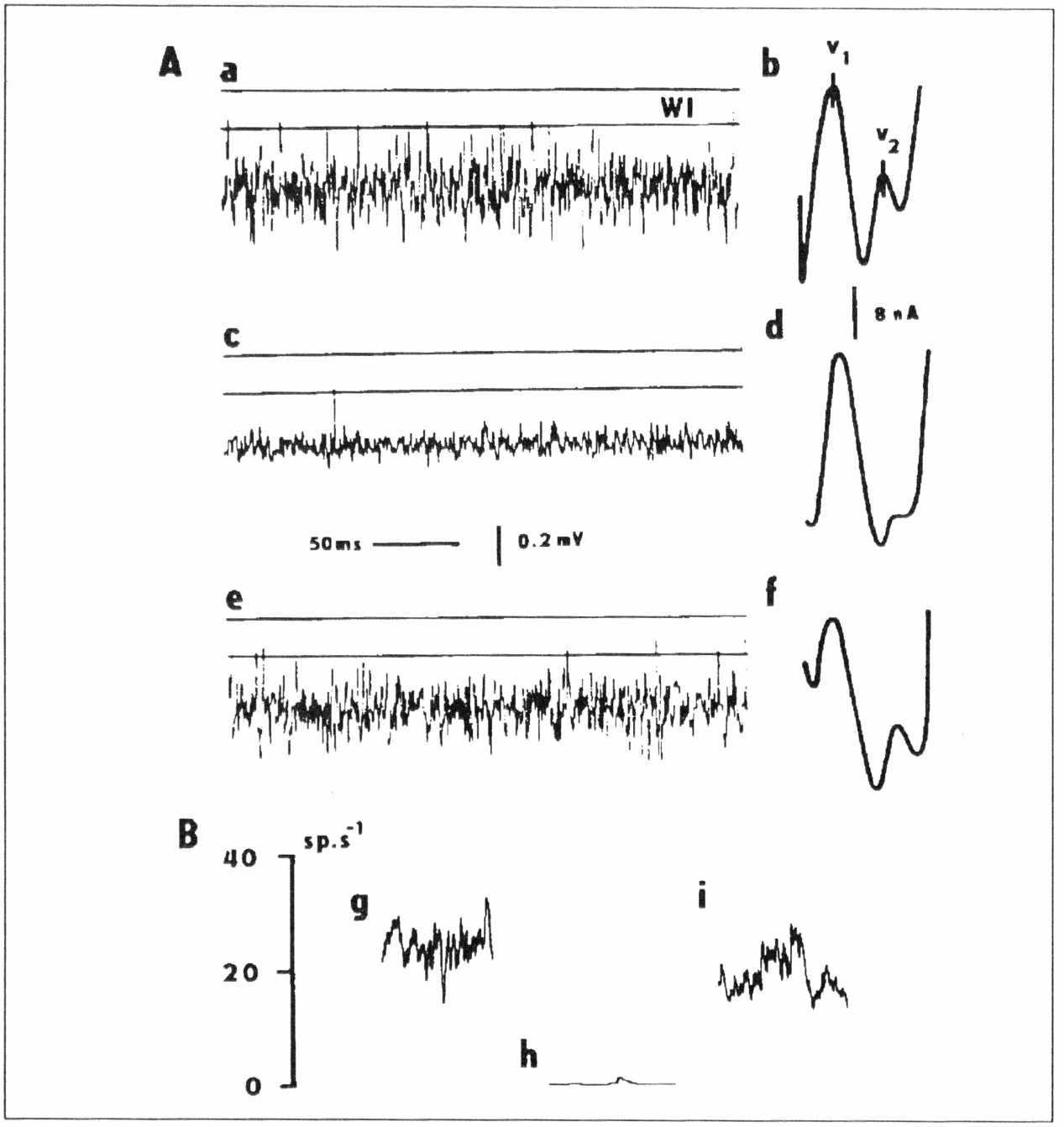

Fig. 2

Effect of the microinjection of KCl on activity of the neurones and on the voltammetric signal in the reticular formation. A. Changes in extracellularly recorded neuronal activity – recordings a (the same as in Fig. 1 D,g), c and e; reactions of the voltammetric signal – recordings b, d and f. a and b – control recordings, c and d – 7 min, e – 9 min and f – 24 min after administering 3 μl 0.5 mol.l^{-1} KCl. Calibration for a, c and e: 50 ms, 0.2 mV. Maximum amplitude of the first (v_1) and second (v_2) peak of the voltammograms was reached at +120 mV and +400 mV (the voltage on the working electrode); the vertical calibration line for b, d and f represents 8 nA. WI – electronic window. B. Extracellular recording of neuronal unit activity – frequency of the incidence of action potentials. (sp.s^{-1}, ordinate) g – control, h – 1 to 3 min after administering KCl, i – 9 to 12 min after. The result of the experiment which is documented in part A.

interval) was greater than in the S (to 95 % with a 3 min interval and to 56 % with a 1 min interval). For this reason, different intervals were used in the experiments for measurement in the S (1 min) and the RF (3 min); the purpose of this choice was to study the dynamics of reactions with a least interval between measurements on the one hand in an attempt to minimize the influence of measurement on the

microenvironment of the active electrode on the other. The results in Tab. 1 also show that the oxidation-reduction current of the electro-oxidizable substance (EOS) formed at the lower voltage (the first wave in the voltammograms) at the 1 min interval disappears completely in the S and in the RF.

The parameters of the working electrode and the given electronic equipment also allowed (in the intervals between recording the voltammetric signal) continuous recording of multiple unit activity (MUA) in the RF. This activity is illustrated in Fig. 1D g,h.

Correlation of changes induced in the activity of reticular neurones and in the voltammetric signal by microinjection of KCl

A microinjection of 3-5 μl 0.5 mol.l^{-1} KCl was administered into the immediate vicinity of the tip of the working electrode 20 s before terminating the interval between recording the voltammetric signal. The MUA recording (which continued while KCl was being administered) showed a significant depression of this activity which started almost at once (2-3 s after the injection) and persisted up to the 6th minute after its administration (Fig. 2A, c). MUA then began to recover and reached the control level again after 9 min (Fig. 2A, e). The dynamics of this process are also documented by the time course of the frequency of the incidence of action potentials (Fig. 2B).

The first recording of the voltammetric signal after the microinjection of KCl was made 30 s after its administration. Compared with the control CA.OC (Fig. 2 A,b, the peak marked v2), a decrease was already evident in this phase. The maximum decrease in the CA.OC (to 26 % of the control) was recorded 6 min after the microinjection (Fig. 2 A,d). From the 9th minute the size of the CA.OC began to increase again and by the 24th minute it attained 70 % of the control value (Fig. 2 A,f); after that the voltammetric signal was not studied any further.

Comparison of the effect of the microinjection of KCl on the catecholamine current in the reticular formation and the corpus striatum

The effect of the microinjection of 0.5 mol/l^{-1} KCl into the RF and S on the CA.OC in these structures was quite different. In the RF there was a decrease in the CA.OC (Fig. 2A, d), which was already evident in the 1st min after administration (mean 59 ± 32 %, n = 4) and reached its maximum (23 ± 27 %, n = 4) 7 min after.

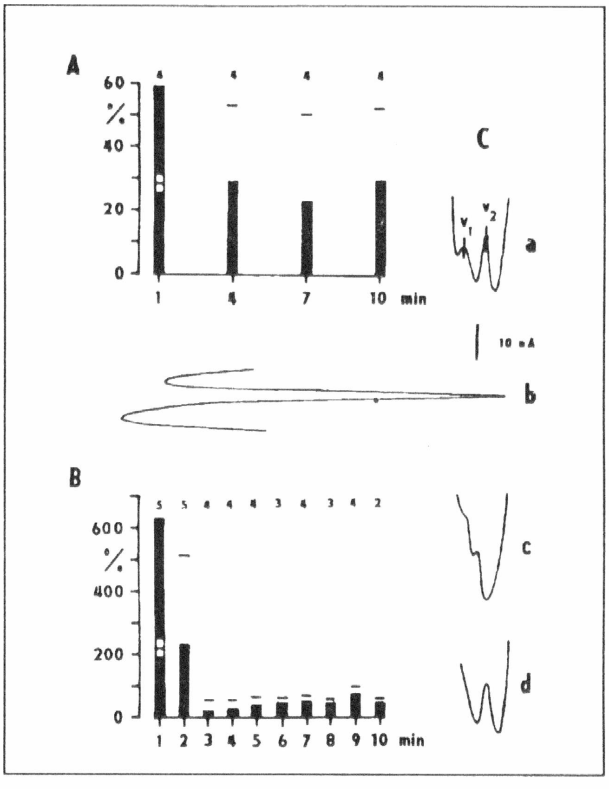

Fig. 3

Effect of the microinjection of KCl on the level of electro-oxidizable substances in the reticular formation and corpus striatum. A. Amplitude changes in the wave representing the reticular formation catecholamine-oxidation current (CA.OC) in the voltammogram. Ordinate: percentage of the control; abscissa: minutes after administering KCl when voltammetric measurement was carried out. The columns are the arithmetical means (the figures above the columns stand for the number of experiments); the horizontal lines show the standard deviation -SD (1) or +SD (the rest). B. Amplitude changes in the wave representing the corpus striatum (S) CA.OC in the voltammogram. Other details as in A. C. Changes in the voltammetric recording from the S after the microinjection of 5 ll 0.5 mol.l^{-1} KCl into the immediate vicinity of the working electrode. a - control (the peak of the v_1 wave was reached at a polarization voltage of +100 mV and of the v_2 wave at +350 mV; b - recording (horizontal) 1 min after administration, c - 3 min after, d - 20 min after. The vertical line (10 nA) calibrates the CA.OC. (Fig. 3A). The size of the CA.OC then began to increase again (at 10 min it was 29 ± 23 %, n = 4) and by the end of the observation interval (24 min after administering KCl, not documented), the CA.OC values amounted to 45-80 % of the control. The response of S to the same microinjection was biphasic in character, however. One min after administering KCl the CA.OC increased (Fig. 3C, b) – the mean value up to 626 ± 409 %, n = 4 (Fig. 3B); at 2 min the increase in the mean values still persisted, but from the 3rd to the 10th min the CA.OC decreased significantly, with a drop in the mean values to 31-63 % of the control (Fig. 3B).

Table 1

Influence of the interval (I) on the wave which appears in the voltammetric recording at the lower polarization voltage (EOS) and on the second wave appearing at the higher polarization voltage (CA.OC). The data are from two experiments (corpus striatum, reticular formation); the values (arithmetical means ± S.D.) are expressed as percentage of the control (see text).

	Corpus striatum		Reticular formation	
I [min]				
	EOS	CA.OC	EOS	CA.OC
5	84.0 ± 15.4	108.0 ± 2	86.0 ± 24	101.6 ± 12
3	12.2 ± 7.4	95.4 ± 0.8	10.4 ± 2.2	84.4 ± 3.2
1	0	56.0 ± 5	0	27.0 ± 7
0.5	0	32.4 ± 11	0	0

Discussion

The source of the CA.OC in the S is the oxidation of catechol type substances released into the microenvironment, mainly from the axonal endings of monoaminergic neurones of the nigrostriatal pathway (Gonon *et al.* 1981, Gonon and Buda 1985, Gonzales-Mora *et al.* 1988). Owing to the sensitivity of DPV for DA (the minimum detectable concentration is $n \times 10^{-7}$ $mol.l^{-1}$, the voltammetrically determined DA values in the S microenvironment ($n \times 10^{-7}$ $mol.l^{-1}$ – Freed 1987) are limit values. The CA.OC wave in the S is thus rather an indicator of the presence of the DA metabolite dihydroxyphenylacetic acid (DOPAC), the concentration of which, in the S microenvironment, is several hundred times greater than the DA concentration; furthermore, its oxidation-reduction potential is very close to the DA potential (Gonon *et al.* 1980). In the RF of the medulla oblongata and pons Varoli, there are groups of neurones forming the monoaminergic system of the RF (Dahlström and Fuxe 1964); in the rat, this chiefly comprises noradrenergic neurones and, to a smaller extent, adrenergic and dopaminergic neurones (Moore 1980). Their axons project mainly outside the region of the RF (Lindvall and Björklund 1978, Moore 1980); greater density of endings of the given types of neurones was observed only in the dorsomedial and ventrolateral part of the medulla oblongata (Smialowska *et al.* 1985, Lambás-Senas *et al.* 1985). It is therefore probable that the CA.OC measured in the RF is the result of oxidation of catechol type substances which find their way into the extracellular space particularly from the bodies and dendrites of neurones of the monoaminergic system (Lambás-Senas *et al.* 1990). The role of the various catechol type substances in the CA.OC can be identified if combined with pharmacological procedures; this, however, may be associated with undesirable side effects of the systemically administered drugs. The microinjection of 33 $\mu mol.l^{-1}$ NA, administered to the tip of the working electrode, led in the voltammogram to the formation of a very large wave over 11 times higher than the amplitude of the CA.OC in the control recording. The shift of the peak of the wave to lower voltage values (+240 mV as against +300 mV in the control recording) can be ascribed to the change produced in the redox potential of the working electrode by an increase in the concentration of the active substance in its vicinity (Justice 1987). The above technique tested the sensitivity of the electrodes and also allowed (assuming a linear functional correlation) semiquantitative determination of the concentration of catechol type substances in the investigated region of RF – the 11-fold increase in the signal after administering a 33 $\mu mol.l^{-1}$ solution meant a concentration of approximately 3 $\mu mol.l^{-1}$ under control conditions. A value close to this one (5.2 μM) was found by means of differential normal pulse voltammetry in the microenvironment of the rostroventral part of the medulla oblongata (Lambás-Seňas *et al.* 1990). As confirmed by experiments in which catecholamines were administered ionophoretically, NA in a concentration of 10^{-6} to 10^{-8} $mol.l^{-1}$ has a regulatory effect on the discharge activity of the RF neurones (Hricovini and Pavlásek 1985) and the neocortex (Armstrong-James and Fox 1983); inhibitory effects were observed in the neocortex in the presence of higher-micromolecular concentrations.

The sudden increase in $[K^+]_e$ after the microinjection of KCl causes a net flux of K^+ into the cells of the CNS, depolarization of neuronal membranes and suppression of the generation of action potentials (Fig. 2 A,c); net intracellular flux of Na^+ and Cl^- ions and a transport of water molecules from the extracellular space into the cells are concomitant

phenomena. In addition, K^+-evoked depolarization activates processes associated with an increase in the intraneuronal calcium concentration ($[Ca^{2+}]_i$) - Nachsen and Blaustein (1980); the increase in the amount of free $[Ca^{2+}]_i$ plays a key role in the release of neurotransmitters, including catecholamines (Drapeau and Blaustein 1983). The K^+ depolarization-evoked increase in the CA.OC in the S is Ca^{2+}-dependent (Murgaš *et al.* 1991), indicating that the increase in the concentration of catechol type substances is due to their release from the vesicular pool. The biphasic course of changes in the CA.OC in the S after the microinjection of KCl (Fig. 3B, C) is probably the result of two contradictory processes – emptying of the transmitter pool in synaptic endings into the extracellular space and simultaneous transport of the transmitter into cells of the CNS, in which the neuroglia plays an important role (Paulsen and Fonnum 1989). The first phase (an increase in the CA.OC) is characterized by a massive release of the transmitter, whereas in the second phase (diminution of the CA.OC below the control level), mechanisms removing the transmitter from the microenvironment preponderate. The different character of the reaction in the RF after microinjection of KCl (only a decrease in the CA.OC – Fig. 2A, d, Fig. 3A) may be due to the smaller total amount of catechol type substances in the given region, which – with reference to their localization (the somata and the dendrites) – do not necessarily belong to the transmitter (vesicular) pool. The slower rate of "filling" of the RF microenvironment by catechol type substances is also indicated by the smaller CA.OC (compared with the S) on using shorter intervals between voltammetric measurements (Tab. 1). In the case of K^+ depolarization, the rate and amount of catechol type substances transported into the neuroglia on the basis of the increase in its metabolic activity probably preponderate over the amount and the rate at which these substances are released into the microenvironment – manifested in a decrease in the CA.OC.

K^+ depolarization is a part of the mechanisms which are activated in structures of the brain when these are exposed to the action of a sufficiently intensive mechanical, chemical or electrical stimulus. The resultant reaction, known as spreading depression (SD), is manifested in a shift of the extracellular potential to negativity and in depression of neuronal activity which spreads like a wave from the site of the stimulus at a rate of roughly 3 $mm.min^{-1}$ (Leao 1944, Zachar and Zacharová 1963, Bureš *et al.* 1974). SD is accompanied by a marked increase in $[K^+]_e$ (from approximately 3 $mmol.l^{-1}$ to about 40 $mmol.l^{-1}$ - Nicholson 1979) and by a simultaneous decrease, in the extracellular space, in the Na^+, Cl^- and Ca^{2+} ion concentration – evidence that selective membrane permeability is impaired during SD and that transmembrane movement of ions occurs in the direction of their electrochemical gradients. In the area where SD is taking place, protracted changes in the rate of metabolism of noradrenaline (Schanberg *et al.* 1968) and of DA (Keller *et al.* 1972) also belong to its biochemical correlates. The increase in $[K^+]_e$ causes depolarization and that in turn increases $[K^+]_e$ – a cycle which effects the spread of SD in brain structures. K^+ depolarization in the course of SD – like the K^+ depolarization evoked by the microinjection of KCl – activates processes leading to neurotransmitter release. In the course of a wave of SD taking place in the S, an increase occurs in this structure in the CA.OC, which is preceded by an increase in $[K^+]_e$ (Moghaddam *et al.* 1987); an increase in the CA.OC was also observed in the course of the SD wave in the rat cerebral cortex (Pavlásek *et al.* 1991, unpublished results). The released catecholamine type transmitter activates processes which can participate in the formation of further concomitant effects of the SD wave, such as a change in the local circulation, the level of metabolic processes and O_2 tension in the brain tissue; it can also be assumed that ion channels are metabolically influenced by the released transmitter.

Our data confirmed differences in KCl-evoked changes in the concentration of catecholamine type substances in the microenvironment of different brain structures. This shows that the "logic" of signalization when the transmitting channel of the extracellular space is utilized does not depend only on the type of transmitter and the target structures, but also on the microarchitecture and characteristics of local metabolic processes. Regional differences in KCl-evoked reactions, such as changes in the extracellular concentration of transmitters, including catecholamine type transmitters – Tossman *et al.* (1986), or the rise and spread of SD (as distinct from the S, SD cannot be evoked in the RF – Bureš *et al.* 1974), indicate that there may be a wide range of modulatory factors.

References

ADAMS R.N.: In vivo electrochemical recording – a new neurophysiological approach. *Trends in Neurosci.* **1**: 160–163, 1978.

ARMSTRONG-JAMES M., FOX K.: Effects of ionophoresed noradrenaline on the spontaneous activity of neurones in rat primary somatosensory cortex. *J. Physiol.*(Lond.) **335**: 427–447, 1983.

BERTOLUCCI-D'ANGIO M., SERRANO A., SCATTON B.: Differential effects of forced locomotion, tail-pinch, immobilization, and methyl- - carboline carbohydrate on extracellular 3,4-dihydroxyphenylacetic acid levels in the rat striatum, nucleus accumbens, and prefrontal cortex: an in vivo voltammetric study. *J. Neurochem.* 55: 1208–1215, 1990.

BUREŠ J., BUREŠOVÁ O., KŘIVÁNEK J.: The Mechanism and Applications of Leao's Spreading Depression of Electroencephalographic Activity. Academia, Prague, 1974, 410 pp.

CHERAMY A., LEVIEL V., GLOWINSKI J.: Dendritic release of dopamine in the substantia nigra. *Nature* 289: 537–542, 1981.

DAHLSTRÖM A., FUXE K.: Evidence for the existence of monoamine-containing neurons in the central nervous system. I.Demonstration of monoamines in the cell bodies of the brain stem neurons. *Acta physiol. scand.* 62 (suppl.232): 1–55, 1964.

DRAPEAU P., BLAUSTEIN P.B.: Initial release of [^{3}H]dopamine from rat striatal synaptosomes: correlation with calcium entry. *J. Neurosci.* **3**: 703–713, 1983.

FAIRBROTHER I.S., ARBUTHNOTT G.W., KELLEY J.S., BUTCHER S.P.: In vivo mechanisms underlying dopamine release from rat nigrostriatal terminals: II.Studies using potassium and tyramine. *J. Neurochem.* **54**: 1844–1851, 1990.

FIFKOVÁ E., MARŠALA J.: Stereotaxic Atlas for the Cat, Rabbit and Rat Brains. Avicenum, Praha, 1960, pp.41–58.

FREED C.R.: In vivo voltammetry and the neurochemical control of movement and blood pressure. In: *Voltammetry in the Neurosciences*. J.B. JUSTICE Jr. (ed), The Humana Press, Clifton, New Jersey, 1987, pp.347–374.

GONON F.G., BUDA M.J.: Regulation of dopamine release by impulse flow and by autoreceptors as studied by in vivo voltammetry in the rat striatum. *Neuroscience* **14**: 765–774, 1985.

GONON F.G., BUDA M., CESPUGLIO R., JOUVET M., PUJOL J.F.: In vivo electrochemical detection of catechols in the neostriatum of anaesthetized rats: dopamine or dopac? *Nature* **286**: 902–904, 1980.

GONON F.G., BUDA M., CESPUGLIO R., JOUVET M., PUJOL J.F.: Voltammetry in the striatum of chronic freely moving rats: detection of catechols and ascorbic acid. *Brain Res.* **223**: 69–80, 1981.

GONON F.G., BUDA M., PUJOL J.F.: Treated carbon fibre electrodes for measuring catechols and ascorbic acid. In: *Measurement of Neurotransmitter Release in Vivo*. C.A. MARDENS (ed), John Willey & Sons, Ltd., London, 1984, pp.153–171.

GONZALES-MORA J.L., SANCHEZ-BRUNO J.A., MAS M.: Concurrent on-line analysis of striatal ascorbate, dopamine and dihydroxyphenylacetic acid concentrations by in vivo voltammetry. *Neurosci. Lett.* **86**: 61–66, 1988.

HÖSLI L., TEBECIS A.K., SCHÖNWETTER H.P.: A comparison of the effects of monoamines on neurones of the bulbar reticular formation. *Brain Res.* **25**: 357–370, 1971.

HRICOVINI M., PAVLÁSEK J.: Desensitization of reticular neurons after ionophoretically applied noradrenalin in rats. *Endocrinol. Exper.* **19**: 291–296, 1985.

JUSTICE J.B.Jr.: Introduction to in vivo voltammetry. In: *Voltammetry in the Neurosciences*. J.B. JUSTICE, Jr. (ed), The Humana Press, Clifton, New Jersey, 1987, pp.3–101.

KELLER H.H., BARTHOLINI G., PIERI L., PLETSCHER A.: Effects of spreading depression on the turnover of cerebral dopamine. *Europ. J. Pharmacol.* **20**: 287–290, 1972.

KRNJEVIC K.: Neurotransmitters in cerebral cortex. A general account. In: *Cerebral Cortex*. Vol. 2. Functional Properties of Cortical Cells. E.G. JONES, A. PETERS (eds), Plenum Press, New York, 1984, pp.39–61.

LAMBÁS-SEÑAS L., CHAMBA G., DENNIS T., RENAUD B., SCATON B.: Distribution of dopamine, noradrenaline and adrenaline in coronal sections of the rat lower brainstem. *Brain Res.* **347**: 306–312, 1985.

LAMBÁS-SEÑAS L., GILLON J.Y., BOUILLOX J.P., SECCIA M., BUDA M., RENAUD B.: In vivo monitoring of catecholaminergic metabolism in the C1 region of rat medulla oblongata: A comparative study by voltammetry and intracerebral microdialysis. *J. Neurochem.* **54**: 2042– 2049, 1990.

LANE R.F., HUBBARD A.T., FUKUNAGA K., BLAUCHARD R.J.: Brain catecholamines: Detection in vivo by means of differential pulse voltammetry at surface-modified platinum electrodes. *Brain Res.* **114:** 346–352, 1976.

LEAO A.A.P.: Spreading depression of activity in the cerebral cortex. *J. Neurophysiol.* **7:** 359–390, 1944.

LINDVALL O., BJÖRKLUND A.: Organization of catecholamine neurons in the rat central nervous system. *Handbook Psychopharmacol.* **9:** 139–231, 1978.

MAY L.J., WIGHTMAN R.M.: Heterogeneity of stimulated dopamine overflow within rat striatum as observed with in vivo voltammetry. *Brain Res.* **487:** 311–320, 1989.

MERMET C.C., GONON F.G.: Ether stress stimulates noradrenaline release in the hypothalamic paraventricular nucleus. *Neuroendocrinol.* **47:** 75–82, 1988.

MOGHADDAM B., SCHENK J.D., STEWARD W.B., HANSEN A.J.: Temporal relationship between neurotransmitter release and ion flux during spreading depression and anoxia. *Can. J. Physiol. Pharmacol.* **65:** 1105–1110, 1987.

MOORE R.Y.: The reticular formation: monoamine neuron systems. In: The Reticular Formation Revisited. J.A. HOBSON, M.A.B. BRAZIER (eds), Raven Press, New York, 1980, pp.67–81.

MURGAŠ K., ORLICKÝ J., PAVLÁSEK J.: Monitoring of potassium-stimulated catecholamine changes in striatal synaptosomal preparations and in corpus striatum of rats: A comparative voltammetric study. *Gen. Physiol. Biophys.* **10:** 421–431, 1991.

NACHSHEN D.A., BLAUSTEIN M.P.: Some properties of potassium-stimulated calcium influx in presynaptic nerve endings. *J. Gen. Physiol.* **76:** 709–728, 1980.

NICHOLSON C.: Brain-cell microenvironment as a communication channel. In: *The Neurosciences, Fourth Study Program.* F.O. SCHMIDT, F.G. WORDEN (eds), The Mit Press, Cambridge/Massachusetts/London, 1979, pp.457–476.

PAULSEN R.E., FONNUM F.: Role of glial cells for the basal and Ca^{2+}- dependent K^+-evoked release of transmitter amino acids investigated by microdialysis. *J. Neurochem.* **52:** 1823–1829, 1989.

PAVLÁSEK J., DEKAN E.: Simple method of microinjections into the brain of wake rats. *Čs. fysiol.* **35:** 430–431, 1986. (in Slovak).

SCHANBERG S.M., SCHILDKRAUT J.J., KŘIVÁNEK J., KOPIN I.J.: Effect of cortical spreading depression on norepinephrine-H^3 metabolism in brain stem. *Experientia* **24:** 909–910, 1968.

SMIALOWSKA M., BAL A., SOLTYS Z., KALUZA J.: Monoamine distribution on the ventral surface of the rat medulla oblongata. *J. Neural Transm.* **63:** 13–29, 1985.

TAUC L.: Nonvesicular release of neurotransmitter. *Physiol. Rev.* **62:** 857–893, 1982.

TOSSMAN U., JONSSON G., UNGERSTEDT U.: Regional distribution and extracellular levels of amino acids in rat central nervous system. *Acta physiol. scand.* **127:** 533–545, 1986.

ZACHAR J., ZACHAROVÁ D.: Initiation of Spreading Cortical Depression (in Slovak). VSAV, Bratislava, 1963, 110 pp.

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