Influence of Hypoxia on Release and Uptake of Neurotransmitters in Guinea Pig Striatal Slices: Dopamine and Acetylcholine

Kiyofumi SAIJOH*, Hiroshi FUJIWARA and Chikako TANAKA**
Department of Pharmacology, Kobe University School of Medicine, Kobe 650, Japan

Accepted September 2, 1985

Abstract—We studied the influence of hypoxia on the release of [3H]dopamine ([3H]DA) and [3H]acetylcholine ([3H]ACh), uptake of [3H]DA and [3H]choline and Ca2+-influx in guinea pig striatal slices. Tetrodotoxin (TTX)-sensitive and Ca2+-dependent electrically evoked release of [3H]DA was not affected by hypoxia, while spontaneous release of [3H]DA was rapidly increased. On the other hand, by hypoxia, the evoked [3H]ACh release gradually decreased and was diminished to about 45% 40 min later. Hypoxia suppressed the Vmax of [3H]DA uptake to one third and that of [3H]choline to half of the control values, but with no change in either of the Km values. Hypoxia reduced both the acetylation and the uptake of [3H]choline in slices preliminarily incubated with 3 mM or 25 mM K+ medium. Stimulation-induced Ca2+-influx was slightly suppressed and was 78.1% of the control values even after 40 min exposure to hypoxia. The Ca2+-dependent neurotransmitter release process itself appears to be well preserved against hypoxia. Our findings imply that hypoxia could result in differential alterations of neural activity depending on the specific sensitivity of the presynaptic process of neurotransmission.

In mammals, severe cerebral hypoxia causes a rapid failure of neurotransmission in vivo and in vitro. The basis for synaptic transmission block by hypoxia was shown to be a depolarization of neuronal membranes and a decline in ATP, most likely induced through inhibition of the Na+/K+ pump (1–3). On the other hand, the early functional deficits due to acute hypoxia are considered to be mediated by impaired presynaptic function in neurotransmission processes rather than measurable changes in energy metabolites (4). The synthesis of acetylcholine (ACh) (5), catecholamines, and serotonin (6) is oxygen-dependent. The uptake of choline and release of ACh are reported to be suppressed by low oxygen and chemical hypoxia, in vivo. However, biochemical studies of neurotransmitters in the hypoxic brain in vivo are limited by the complexity of the central nervous system. An approximation of in vivo hypoxia was studied using synaptosomes, brain slices and peripheral tissue strips. Gibson and Peterson (7) reported that hypoxia reduces the Ca2+-dependent ACh release, in parallel with the synaptosomal Ca2+ uptake (8). On the other hand, hypoxia induced an increased spontaneous release of ACh from brain synaptosomes (9). The mechanisms involved in the selective sensitivity of the cholinergic nerve system to low oxygen and the link between the hypoxia-induced alterations in transmitter release and the intracellular free Ca2+ remained unclear. We have reported that the uptake and acetylation of choline in newborn guinea pig brains are low, and the sensitivities to hypoxia differ with the brain region (10). In the present paper, in attempts at elucidation of the pathophysiological mechanisms of brain damage in the presence...
of hypoxia, we examined the effect of low oxygen on release of \[^{3}H\]dopamine (\[^{3}H\]DA) and \[^{3}H\]ACh on uptake of \[^{3}H\]DA and \[^{3}H\]choline and the intracellular Ca\(^{2+}\) level in guinea pig striatal slices.

**Materials and Methods**

**Striatal slices:** Guinea pigs of both sexes weighing 350–450 g were decapitated, the brains quickly removed and cut coronally at the level of the anterior commissura. The striatal slices were dissected with a razor blade and a sliding guide. The caudal part of the striatum and globus pallidus was excluded. Frontal sections used in the experiments were 350–450 \(\mu\)m thick and weighed 6–8 mg. For the uptake experiments, the slices were further chopped to pieces about 1.5 mg in weight.

**Incubation medium:** A Krebs Ringer solution buffer, pH 7.4, containing 118 mM NaCl, 3 mM KCl, 2 mM CaCl\(_2\), 1.2 mM MgCl\(_2\), 1.2 mM NaH\(_2\)PO\(_4\), 23 mM NaHCO\(_3\), 11 mM glucose, 0.01% ascorbate, and 0.002% pargyline was equilibrated with 95% O\(_2\) 5% CO\(_2\) (control medium: O\(_2\) tension, about 530 mmHg) or 95% N\(_2\) 5% CO\(_2\) (hypoxic medium: about 30 mmHg). When tissues were perfused with the hypoxic medium, oxidative metabolism was measured by pyridine nucleotide fluorescence (1) and evoked field potentials in slices measured by the method of Misgeld et al. (11). Oxidative metabolism was impaired in parallel with the decrease in O\(_2\) tension, and amplitudes of the negative field potentials (N-2) were abolished within 5–10 min (data not shown).

For the hypoxic preparation, we maintained the O\(_2\) tension to 30 mmHg in all hypoxic experiments. In some experiments, Ca\(^{2+}\)-free medium was prepared. The composition of this "Ca\(^{2+}\)-free medium" was as above except: 0 mM CaCl\(_2\), 122 mM NaCl and 10\(^{-4}\) M EGTA.

**\[^{3}H\]DA and \[^{3}H\]ACh release from striatal slices:** Slices were preloaded with \[^{3}H\]DA or \[^{3}H\]choline (10\(^{-7}\) M) for 1 hr at 37°C and then rinsed with the control medium. The preparation was impaled with a pair of parallel platinum electrodes (0.1 mm in diameter; distance, 0.7 mm; length, 1.5 mm), mounted in the superfusion chamber saturated with 95% O\(_2\) – 5% CO\(_2\) or 95% N\(_2\) – 5% CO\(_2\) and superfused at a constant flow rate of 0.2 ml/min with control or hypoxic medium at 37°C, respectively (12). The superfusate was continuously collected every 5 min and radioactivity determined in a liquid scintillation spectrometer. The hypothetical decline curve of spontaneous release was calculated according to the formula \(y = Ae^{-at}\), \(y\): log (released count: cpm), \(t\): min. The constants A and B were calculated by the release from 10 to 40 min, under both control and hypoxic conditions. Electrical stimulation of striatal slices was performed under conditions of various currents and frequencies. The evoked release was calculated by the formula: (the stimulated release)/(the spontaneous release) – 1.

**Unchanged \[^{3}H\]DA and \[^{3}H\]ACh in the superfusate and slices were determined as follows:** Superfusates from electrically stimulated slices, before and during stimulation, were collected for 5 min. Electrical stimulations of 1 mA, 1 Hz, 1 msec for 2 min for \[^{3}H\]DA release and of 0.75 mA, 0.5 Hz, 1 msec for 2 min for \[^{3}H\]ACh release were performed at 15 and 40 min after start of superfusion. For the determination of DA, 10 ng of unlabeled DA was added to the sample as the carrier, and the DA in the sample was absorbed with activated alumina, eluted with 0.1 N HCl and identified by HPLC. The fractions containing DA were collected from the liquid scintillation spectrometer (13). For extraction and separation of \[^{3}H\]ACh and \[^{3}H\]choline, the superfusates were collected in 1 ml of 3-heptanone-tetraphenylboron (10 mg/ml) on ice. \[^{3}H\]ACh and \[^{3}H\]choline were extracted with HCl, dried, dissolved in 1 N formic acid-acetone (15:85, v/v), and separated by the electrophoretic method (10). Recovered radioactive compounds on dried paper strips were extracted with 0.5 ml of ethanol, and the radioactivity measured in a toluene base scintillator, using a liquid scintillation spectrometer.

**\[^{3}H\]DA uptake:** Striatal slices were pre-incubated for 15 min at 37°C in 50 ml of control or hypoxic medium bubbling with 95% O\(_2\) – 5% CO\(_2\) or 95% N\(_2\) – 5% CO\(_2\), respectively. For kinetic determination, the slices were incubated in 5 ml of each medium
containing [3H]DA (0.1 to 2 μM) for 10 min at 37°C. After incubation, tissues were washed with 50 ml of fresh medium twice every 10 min at 0°C and then dissolved by sonication in 0.5 ml of sodium laurylsulfate (10%). The total radioactivity in the tissues was measured by a liquid scintillation spectrometer. The samples incubated at 0°C served as blanks to the 37°C samples.

**Uptake and acetylation of [3H]choline:**
In order to activate the high affinity choline uptake, the 25 mM K+ medium (K+ replacing Na+ isoosmotically) was used. Striatal slices were preliminarily incubated for 15 min at 37°C in 50 ml of control (3 mM K+ or 25 mM K+) or hypoxic (3 mM K+ or 25 mM K+) medium. For kinetic determination, tissues were rinsed twice within 1 min with 5 ml control or hypoxic medium and then incubated for 5 min in 10 ml control or hypoxic medium containing [3H]choline (0.1 to 10 μM). The low volume of the slices was used to minimize the concentration of endogenous choline in the incubation medium. The total radioactivity in the tissues was measured.

Acetylation of [3H]choline was measured in the slices incubated with each medium containing 0.2 μM [3H]choline. After incubation, tissues were washed and then homogenized in 20 vol. of formic acid-acetone. [3H]ACh and [3H]choline extracted from tissues were separated by electrophoresis.

**Calcium influx:** Using the method of Tsien et al. (14), slices were loaded by incubation with 1 mM quin2/AM and 0.5% DMSO (v/v) in control medium without CaCl2, at 37°C for 1 hr and washed 3 times with the control medium. The preparation was impaled with a pair of parallel platinum electrodes (0.1 mm in diameter; distance, 2.5 mm; length, 3 mm) and then mounted vertically in the cuvette in 1 ml of control or hypoxic medium and perfused at a constant flow rate of 3.5 ml/min at 37°C, and the fluorescence of the quin2-Ca complex was recorded using an Aminco-Bowman spectrophotometer setting with 339 nm excitation, 2 nm slits and 492 nm emission, 3 mm slits. Relative intensity of fluorescence was determined under control and hypoxic conditions, with or without electrical stimulation of 4 mA, 2 Hz, 3 msec for 8 sec. The intensity of fluorescence rapidly declined during the first 30 min perfusing period, but after 60 min, it very slowly declined. Sixty min later on, the evoked increase in the intensity of quin2-Ca complex fluorescence was stable. The experiment was therefore performed during the 2nd–3rd hr. The Co2+ medium was prepared in order to block the Ca2+-channel. The composition of this "Co2+ medium" was 112 mM NaCl, 3 mM KCl, 5 mM CoCl2, 1.2 mM MgCl2, 1.2 mM NaH2PO4, 23 mM HEPES and 11 mM glucose and bubbling with 100% O2.

**Drugs and chemicals:** Drugs used included: [3H]DA (20.1 Ci/mmol) and [3H]choline chloride (80.1 Ci/mmol) from New England Nuclear, dopamine HCl and pargyline HCl (Sigma), tetrodotoxin (Sankyo), benztrapine (Merck-Banyu), hemicholinium-3 (Aldrich), quin2/AM (Wako), A23187 (Calbiochem-Behring). All other chemicals were of reagent grade.

**Results**

**Effect of hypoxia on release of [3H]DA:** Electrical stimulation of striatal slices pre-loaded with [3H]DA produced an increase in the tritium efflux. The amounts of unchanged [3H]DA in the sample obtained before and during stimulation were more than 90 and 95% of the total radioactivity, respectively, under both control and hypoxic conditions. Accordingly, the total radioactivity in the superfusate will further be denoted as [3H]DA release. The [3H]DA release was dependent on the frequency, 1 to 20 Hz, (Fig. 1 A) and current, 0.1 to 2 mA, (Fig. 1 B) of the electrical stimulation. As shown in Fig. 1 B, the release of [3H]DA with stimulation of less than 2 mA was inhibited when the slices were superfused with media containing 10-7 M tetrodotoxin (TTX) or with Ca2+-free medium, indicating a evoked release from dopaminergic terminals. The hypothetical decline curve of spontaneous efflux of [3H]DA was calculated to be \( y = 788e^{-0.0078t} \) under control conditions and \( y = 1053e^{-0.00671t} \) under hypoxic conditions, indicating that hypoxia produced a 30% increase in the spontaneous release of [3H]DA. This increase of spontaneous release was rapidly diminished after the hypoxic
medium was replaced with the control medium (Fig. 2). Under the hypoxic condition, the stimulated release was also increased (Fig. 2) so that the evoked release of [3H]DA was not significantly changed (Fig. 3A). As shown in Fig. 4A, the presence of 10^{-6} M of benztropine (about twice as much as the IC50 value), a specific DA uptake blocker (15), in the superfusion medium led to an increase in the evoked release of [3H]DA to 118% of the control 15 min later and more than 140% after 40 min, without apparent change in the spontaneous release, while spontaneous release was increased to about twice the control values by 10^{-5} M benztropine, which is reported to induce a true releasing effect at that concentration (16).

Effect of hypoxia on release of [3H]ACh: The tritium efflux was increased in response to electrical stimulation from striatal slices preloaded with [3H]choline. The efflux was dependent on the frequency, 0.1 to 1 Hz (Fig. 1A), and current, 0.1 to 2 mA (Fig. 1C), of the electrical stimulation. The release of [3H]ACh induced by a stimulation of less than 2 mA was markedly inhibited by adding 10^{-7} M TTX to the superfusion medium or by Ca^{2+}-free medium, indicating a evoked release from cholinergic nerve terminals (Fig. 1C). The amounts of [3H]ACh in the sample obtained before and during stimulation were 72±8 and 81±6% for control, 72±7 and 74±5% for 15 min hypoxia, and 66±4 and 66±5% for 40 min hypoxia. The amount of [3H]ACh release was calculated according to the percent described above. As shown in Fig. 3B, the evoked release of [3H]ACh was decreased about 45% when the slices were
superfused with hypoxic medium for 40 min, and this decrease in evoked release was returned to the control level by superfusing with the control medium for 40 min, without significant change in spontaneous release. Addition to the superfusing medium of hemicholinium-3 of 10^{-6} M (4-fold of the Ki value), a specific blocker of choline uptake (17), produced an increase in evoked release to 168% without a significant alteration in spontaneous release. Addition of 10^{-5} M of hemicholinium-3 produced a transient increase in spontaneous efflux of [3H]ACh, while the evoked release of [3H]ACh was decreased to about 80% after 40 min (Fig. 4B).

Effect of hypoxia on uptake of [3H]DA: The high affinity uptake of DA and choline are well-known to be energy-dependent. To ascertain the possible involvement of the uptake suppression by hypoxia to the transmitter release, we determined the effect of hypoxia on uptake of DA and choline in the following experiments.

Accumulation of [3H]DA into striatal slices increased linearly for 10 min and reached a plateau 30 min later. When slices were incubated with [3H]DA in concentrations ranging from 0.1 to 2 \mu M at 37°C for 10 min, under oxygenated conditions, the radioactivities in the slices were increased, in a dose-dependent manner. Double reciprocal plots of initial velocities of [3H]DA uptake and concentrations of [3H]DA in the medium gave a single component, with a K_m value of 6.96±0.32\times10^{-7} M and a V_max value of 15.53±0.66 pmol/mg/10 min. In slices incubated with hypoxic medium, the V_max value decreased to 5.45±0.75 pmol/mg/10 min, without significant change in the K_m value (Table 1).

Effect of hypoxia on uptake and acetylation of [3H]choline: The amount of [3H]choline taken up by the slices increased linearly for 5 min and to a maximum 20 min later. Slices were incubated in the control medium containing [3H]choline of varying concentrations from 0.1 to 10 \mu M at 37°C for 5 min. A double reciprocal plot for [3H]choline accumulation in the striatum yielded a single component with K_m of 1.42±0.20 \mu M and V_max of 3.30±0.46 pmol/mg/5 min. In slices incubated with the hypoxic medium, the V_max value was decreased to 1.40±0.07 pmol/mg/5 min, whereas the K_m value remained unchanged. High K^+, 25 mM, medium produced an increase in the V_max value to 5.65±0.51 pmol/mg/5 min without
any alteration in the $K_m$ value. In slices treated with the high $K^+$ medium, hypoxia induced a decrease in the $V_{\text{max}}$ value to about 75% of the control values (Table 1). Hypoxia had no effect on the uptake in slices incubated with more than 20 nM $[^3H]$choline (data not shown).

As shown in Fig. 5, in the slices incubated with control medium containing 0.2 nM of $[^3H]$choline for 5 min, the total $[^3H]$choline accumulating in the slices was 384±33 fmol/mg/5 min, and 62.7% of the total tritium was acetylated (241±25 fmol/mg/5 min). The synthesis of ACh was decreased to 27.4% under hypoxic conditions, while the total uptake of $[^3H]$choline decreased to 37.0%. Under aerobic conditions, the high $K^+$ produced increases in uptake of $[^3H]$choline to 691±59 fmol/mg/5 min and in acetylation to 80.0% (553±72 fmol/mg/5 min). In these slices, hypoxia induced decreases in total tritium accumulation to 41.6% and in acetylation to 32.2%.

Effect of hypoxia on $Ca^{2+}$-influx: Inhibition of ACh release was reported to be caused by suppression of $Ca^{2+}$-influx under hypoxia (8). Our results indicate that inhibition of ACh release was mainly due to inhibition of
synthesis of ACh, and Ca\(^{2+}\)-dependent DA release was not affected by hypoxia. In order to clarify the discrepancy, we next determined the effect of hypoxia on Ca\(^{2+}\)-influx using quin2 loaded slices.

As shown in Fig. 6A, electrical stimulation produced a transient increase in the fluorescence signal of the quin2-Ca complex.

### Table 1. Effect of hypoxia on uptake of \([3^H]\)DA and \([3^H]\)choline

<table>
<thead>
<tr>
<th></th>
<th>(K_m)</th>
<th>(V_{\text{max}})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>((\times 10^{-7} \text{ M}))</td>
<td>((\text{pmol/mg/10 min}))</td>
</tr>
<tr>
<td><strong>DA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 3 mM K(^+)</td>
<td>6.96±0.32</td>
<td>15.53±0.66</td>
</tr>
<tr>
<td>Hypoxia 3 mM K(^+)</td>
<td>7.19±0.99</td>
<td>5.45±0.75*</td>
</tr>
<tr>
<td><strong>Choline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 3 mM K(^+)</td>
<td>1.42±0.20</td>
<td>3.30±0.07</td>
</tr>
<tr>
<td>Hypoxia 3 mM K(^+)</td>
<td>1.66±0.22</td>
<td>1.40±0.07*</td>
</tr>
<tr>
<td>Control 25 mM K(^+)</td>
<td>1.38±0.20</td>
<td>5.65±0.51*</td>
</tr>
<tr>
<td>Hypoxia 25 mM K(^+)</td>
<td>1.43±0.22</td>
<td>2.47±0.12*</td>
</tr>
</tbody>
</table>

Slices were preincubated with the control (3 mM K\(^+\) or 25 mM K\(^+\)) or hypoxic (3 mM K\(^+\) or 25 mM K\(^+\)) medium for 15 min and then incubated with the control or hypoxic medium containing various concentrations of \([3^H]\)DA for 10 min and \([3^H]\)choline for 5 min. Each value was the mean±S.E. of 6 determinations. Significantly different from control 3 mM K\(^+\) values, *\(P<0.001\); from control 25 mM K\(^+\) value, #\(P<0.001\).
repeatedly. This increase was abolished by the Ca²⁺-free medium, by the TTX (10⁻⁷ M) containing medium and by the Co²⁺ medium. A Ca²⁺-sensitive increase in the fluorescence was also induced by adding 1 μM A23187, a Ca²⁺-ionophore (Fig. 6B). From these findings, the increase in the intensity of fluorescence was mainly due to the increment in intracellular Ca²⁺ due to the increase in the Ca²⁺-influx. The electrically stimulated Ca²⁺-influx was gradually decreased when the slices were perfused with the hypoxic medium and was reduced to 78.1% of the control value after 40 min exposure to a hypoxic condition (Table 2).

**Table 2. Effect of hypoxia on stimulated calcium influx**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th></th>
<th>Hypoxia</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>10 min</td>
<td>20 min</td>
<td>30 min</td>
<td>40 min</td>
<td></td>
</tr>
<tr>
<td>Relative fluorescence intensity (arbitrary units)</td>
<td>35.1±3.1</td>
<td>30.1±2.3</td>
<td>28.6±2.5</td>
<td>30.0±2.7</td>
<td>29.3±2.2</td>
<td>27.5±1.0*</td>
</tr>
<tr>
<td>% of control</td>
<td>85.5</td>
<td>81.2</td>
<td>88.4</td>
<td>83.2</td>
<td>78.1</td>
<td></td>
</tr>
</tbody>
</table>

Slices were electrically stimulated as in Fig. 6A. Amplitude of relative fluorescence intensity was recorded under control or hypoxic condition, at the times shown after start of the hypoxic perfusion. Each value is the mean±S.E. of 6 determinations. Significantly different from the control value. *P<0.05.

**Discussion**

This study revealed that the stimulus-evoked release of DA and ACh was well preserved for at least 15 min after initiation of the superfusion with hypoxic medium, even when the evoked potential was abolished and the uptake of DA and choline was markedly suppressed. Forty min later, however, the spontaneous release of DA was significantly increased, while the stimulation-evoked release of DA was not significantly changed by the hypoxic superfusion. On the other hand, hypoxia markedly suppressed the evoked ACh release, but not the spontaneous...
ACh release from striatal slices.

A possible mechanism for hypoxia-induced changes on neurotransmitter release is an inhibition of re-uptake of the transmitter or its precursor into nerve terminals. We next noted that the uptake of DA and choline into the dopaminergic and cholinergic nerve terminals was susceptible to hypoxia. Our results indicate that kinetic constants for uptake of DA and choline are characteristic of the high affinity uptake system, which is in good agreement with the values reported by other authors (18, 19). It is interesting to note that hypoxia reduced the $V_{\text{max}}$ values of $[^3\text{H}]$DA uptake to one-third of the control value and that of $[^3\text{H}]$choline uptake to half, while the $K_m$ value of both $[^3\text{H}]$DA and $[^3\text{H}]$choline uptake was not significantly changed. There are at least three different mechanisms related to the inhibition of uptake of DA or ACh. One is competitive inhibition in which $K_m$ values are reduced without changes in the $V_{\text{max}}$ values such as the inhibition of DA uptake induced by benztropine (15) or that of choline uptake induced by hemicholinium-3 (17, 20). Another is the non-competitive inhibition which decreases $V_{\text{max}}$ values without alteration of $K_m$ values such as that of DA uptake into striatal synaptosomes induced by low Na+, low K+ and ouabain (21) or that of monoamines or choline into brain synaptosomes after in vivo treatment with 6-hydroxyDA (22) or acute tract lesion (23). The other mechanism is the non-competitive inhibition which decreases $V_{\text{max}}$ and $K_m$ values such as that of DA uptake induced by high K+ or veratridine (21). The preliminary incubation of slices under depolarizing conditions resulted in activation of high affinity choline uptake, with an increase in the $V_{\text{max}}$ value (the activated uptake), which is in good agreement with studies of other authors (24, 25). As was seen in the case of the non-activated choline uptake, hypoxia reduced the $V_{\text{max}}$ value of the activated choline uptake without any change in the $K_m$ value. The uptake of $[^3\text{H}]$DA and $[^3\text{H}]$choline seems to be inhibited in case of hypoxia, in a non-competitive manner and with no alteration of $K_m$ values, as was seen with low Na+, low K+ and ouabain. The changes in the $V_{\text{max}}$ value could be due to a change in the number of transport sites or a change in the turnover rate of transport. These findings suggest that the inhibition of DA or choline uptake is due to an inhibition of the Na+ and energy-dependent uptake mechanism induced by the blocking of Na+-K+ ATPase rather than to that induced by membrane depolarization.

ACh synthesis was significantly increased when the high affinity uptake process was activated. This is in good agreement with studies of other authors (26, 27). Hypoxia decreased the rate of synthesis and the amount of choline uptake in slices both non-activated and activated by 25 mM K+. Thus, the present results support the possibility that the high affinity choline uptake is rate limiting and regulatory for the synthesis of ACh and has the capacity to change with neuronal activity. It seems that acetylCoA availability is regulatory for the rate of acetylation as well.

The changes in the release of DA by hypoxia are most likely due to a partial blocking of the reuptake mechanism of DA, because the uptake of DA was inhibited to a third of the control value in the case of 15 min hypoxia, as described above. As shown in Fig. 4A, when DA uptake into nerve terminals was blocked competitively by benztropine, the evoked release of DA was increased, without a significant change in the spontaneous release. The increased spontaneous Ca2+-insensitive DA release may, to a certain extent, be explained by the non-competitive inhibition of DA uptake caused by hypoxia. In the present study, the intracellular free Ca2+ level was first measured as the fluorescence of the quin2-Ca complex in slices preloaded with quin2/AM. The stimulation-induced Ca2+-influx into the slices was suppressed to 78% by hypoxia after 40 min exposure. Suppression of both the Ca2+-influx and the reuptake of DA, concomitantly induced by hypoxia, seemed to have no apparent effect on the stimulation-induced release of DA.

On the other hand, hypoxia induced a significant suppression in the evoked ACh release, but not in the spontaneous ACh release from striatal slices. Hypoxia also
inhibited synthesis of ACh from choline in striatal slices, as was found in the rat brain exposed to hypoxia in vivo (4, 5). This, as well as the inhibition of uptake of choline, a precursor of ACh, and Ca2+-influx, may be responsible for inhibition of the evoked Ca2+-sensitive ACh release, as noted in synaptosomes (8). There is a critical difference between effects of hypoxia on DA and ACh release; i.e., the choline uptake process is rate limiting for the synthesis of ACh (20, 23) and its inhibition results in a reduction of ACh release, while the inhibition of DA uptake into presynaptic nerve terminals causes an increase in the spontaneous release of DA.

Thus, the Ca2+-dependent neurotransmitter release process itself is relatively well preserved against hypoxia. Our findings imply that hypoxia could result in differential alterations of neural activity depending on the specific sensitivity of the presynaptic process of neurotransmission.

Acknowledgements: This work was supported by grants from the Ministry of Education, Science and Culture and the Ministry of Health and Welfare, Japan. We thank Prof. Y. Okada, Department of Physiology, Kobe University, School of Medicine for criticism of the manuscript and M. Ohara of Kyushu University for critical readings. This paper is part of a dissertation submitted by K. Saijoh to Kobe University, School of Medicine, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

References

1 Lipton, P. and Whittingham, T.S.: The effect of hypoxia on evoked potentials in the in vitro hippocampus. J. Physiol. (Lond.) 287, 427–438 (1979)

2 Lipton, P. and Whittingham, T.S.: Reduced ATP concentration as a basis for synaptic transmission failure during hypoxia in the in vitro guinea pig hippocampus. J. Physiol. (Lond.) 325, 61–65 (1982)


