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Aglycemia and ischemia depress spinal synaptic transmission via inhibitory systems involving NMDA receptors

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Abstract

The effects of in vitro aglycemia (glucose-free) and ischemia (glucose-free and O_2-free) were examined on the dorsal root-evoked ventral root spinal monosynaptic and polysynaptic reflexes in neonatal rat spinal cords. Aglycemia and ischemia depressed the reflexes in a time-dependent manner and abolished them by 35 min. The depression by ischemia began immediately while that by aglycemia began after 15 min. The NMDA receptor antagonist, DL-2-amino-5-phosphonovaleric acid (APV), blocked the depression induced by aglycemia completely and that by ischemia partially. Strychnine ($glycine_A$ receptor antagonist) or bicuculline ($GABA_A$ receptor antagonist) blocked the aglycemia-induced depression of the reflexes. In the case of ischemia, strychnine but not bicuculline, blocked the depression partially. The results indicate that aglycemia and ischemia depress the synaptic transmission involving NMDA receptors. Aglycemia involves both γ-aminobutyric acid-ergic and glycinergic inhibitory transmission while ischemia involves other additional mechanisms.

Keywords: Spinal synaptic transmission; Anoxia; Glutamate; Inhibitory transmission; Creatine phosphate

1. Introduction

Different forms of anoxia such as hypoxia (O_2 lack), aglycemia (glucose lack), ischemia (combination of both hypoxia and aglycemia) or chemical anoxia (lack of cellular utilization) ultimately lead to ATP deficiency (Santos et al., 1996; Pringle et al., 1997). The energy, or ATP, is required for the maintenance of membrane polarity, presynaptic and postsynaptic functions. The magnitude of ATP deficiency and the severity of the effects differ with different anoxic conditions (Santos et al., 1996; Pringle et al., 1997; Luhmann and Heinemann, 1992). The decrease of ATP and the magnitude of synaptosomal membrane depolarization were greater in ischemia than in aglycemia (Santos et al., 1996). Similarly, the quantity of glutamate released is much greater in ischemia than under other anoxic conditions. The glutamate so released increases the intracellular Ca^{2+} and produces excitotoxicity (Mattson et al., 1993; Stys, 1998). The neuronal toxicity induced by anoxic insults is shown to involve mechanisms in addition to glutamate excitotoxicity (Stys, 1998). This is amply supported by findings such as excessive release of other transmitters (5-hydroxytryptamine, norepinephrine, dopamine, γ − aminobutyric acid, etc.), reverse operation of Na^{+}–Ca^{2+} exchanger system, generation of free radicals, cellular damage, etc. (Globus et al., 1988; Santos et al., 1996; Stys, 1998). However, in hypoglycemia there was neither the release of additional transmitters nor the associated cellular damage (Santos et al., 1996; Pringle et al., 1997). In contrast, decreased GABA levels have been reported in hypoglycemia as compared to hypoxia (Madl and Royer, 2000). Whatever the case may be, hypoglycemic coma is a known entity where the loss of neuronal function is explicit. This study was therefore undertaken to address the influence of aglycemia (glucose-free conditions) on spinal synaptic transmission and to compare its effect with that of ischemia (glucose-free + O_2-free). The in vitro spinal cord preparation described elsewhere was chosen to elicit the reflexes involving Ia-α motoneuron synapse, where glutamate is a putative transmitter (Jahr and Yoshioka, 1986). In addition, the spinal cord also contains inhibitory and additional excitatory

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neuronal networks (Jahr and Yoshioka, 1986; Deshpande and Warnick, 1988; Deshpande et al., 1987; Wang and Dun, 1990; Elliott and Wallis, 1992; Wallis and Wu, 1993; Warnick et al., 1993).

2. Materials and methods

2.1. Animals, anesthesia and dissection

All the experiments were performed according to the guidelines of the Institute of Medical Sciences, Banaras Hindu University, Varanasi for conducting animal studies.

The rat pups (4–6 days) of either sex (Charles–Foster strain) were anaesthetized with diethyl ether. The vertebral column was removed quickly and placed in a Sylgard-bottomed petri dish containing physiological solution bubbled with 100% oxygen. The spinal cord was dissected out carefully, hemisected sagitally, and then transferred to a small Plexiglass bath (volume < 1 ml) superfused continuously with the oxygenated physiological solution (3–5 ml/min) at 25 ± 0.5 °C. The physiological solution did not contain Mg^{2+} and had the following composition (in mM): NaCl, 124.0; KCl, 5.0; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 4.5 and glucose, 11.0. The pH of the solution was 7.3 after bubbling with O_2. A suction electrode was prepared by fire-polishing a glass capillary tube to the size of a dorsal or ventral root. The diameter of the tube was adjusted in such a way that it could hold the root tightly. The corresponding dorsal and ventral roots between L_{3-5} were sucked into the suction electrodes (filled with physiological solution) for stimulating and recording purposes, respectively, as mentioned earlier (Deshpande et al., 1987; Deshpande and Warnick, 1994; Singh et al., 2002).

2.2. Stimulation and recording

The stimulation of a dorsal root with rectangular pulses, (0.5 ms duration at 0.1 Hz) evoked reflex potentials at the corresponding segmental ventral root between L_{3-5} segments. The stimulating voltage was adjusted to obtain the maximal response (40–50 V). The reflex potentials were amplified (Harvard AC/DC preamplifier), monitored on an oscilloscope, digitized and stored in a personal computer using an A–D card (PCL-208 from Dynalog India).

2.3. Experimental protocol

The preparation was allowed to stabilize for 2–3 h. The averaged signal of 5–6 reflex potentials was recorded. Aglycemia was produced by superfusing the cord with glucose-free physiological solution bubbled with 100% O_2. Ischemia was produced by superfusing the aglycemic solution without O_2 bubbling and hypoxia was produced by superfusing the standard physiological solution without O_2 bubbling. Under both conditions, the pH of the solution was monitored and was around 7.3. In a typical experiment, after recording of the initial reflex response, the cord was exposed to either of the anoxic conditions till abolition of the reflex or up to 120 min, whichever was earlier. At the end, the cord was washed with physiological solution bubbled with O_2 for 30 min. The dorsal root was stimulated continuously at 0.1 Hz and reflex activity was recorded at 5- to 15-min intervals.

In separate groups, after the initial recordings, the cords were superfused with physiological solution containing DL-2-amino-5-phosphonovaleric acid (APV; 10 μM)/bicuculline (1 μM)/strychnine (1 μM) for 30 min and recordings were made. Subsequently, the cords were exposed to aglycemic or ischemic solutions in the presence of APV/bicuculline/strychnine.

2.4. Drugs and solutions

APV was obtained from Tocris Cookson Bristol, UK. Bicuculline methiodide, strychnine hydrochloride and creatine phosphate were obtained from Sigma, St. Louis, MO, USA. Creatine phosphate (10 mM) was added to the aglycemic solution at the time of experimentation. The stock solutions of all the chemicals were prepared in distilled water.

2.5. Statistical analysis

The time–response data were normalized to the initial response and are presented as the means ± S.E.M. The differences between groups were compared by one-way or two-way analysis of variance (ANOVA) or Student's t test (paired or unpaired) as appropriate. A P value < 0.05 was considered significant.

3. Results

More than two potentials were obtained at the ventral root after stimulating the corresponding dorsal root (response at '0' in Figs. 1 and 2). The potential appearing early had a latency of 4.6 ± 0.3 ms and was considered as a monosynaptic reflex. The potential following it had a latency of 12.1 ± 0.6 ms and was considered as a polysynaptic reflex. The mean amplitudes were 2.9 ± 0.5 and 0.5 ± 0.1 mV for monosynaptic reflex and polysynaptic reflex, respectively. The magnitude of the reflex potentials remained unaltered during the observation period of 120 min in the control situation (Figs. 1 and 2).

3.1. Anoxic conditions depressed the spinal reflexes

Superfusion of aglycemic solution abolished both reflexes within 35 min (Fig. 1). In the first 15 min after superfusion of the aglycemic solution, the reflexes remained almost similar to those of the time-matched controls (P>0.1,

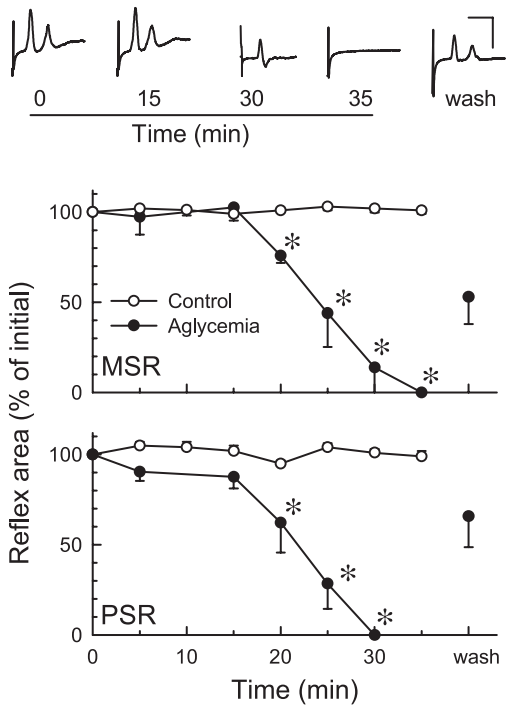

Fig. 1. Aglycemia depressed the spinal reflexes. The upper panel shows the actual discharges of the reflex potentials at different time intervals after superfusion of the aglycemic (glucose-free) solution. Vertical calibration = 1 mV and horizontal calibration = 10 ms. The lower graphs show the time–response values (means ± S.E.M.; $n = 6$) of monosynaptic reflex (MSR) and polysynaptic reflex (PSR) after exposure to aglycemic solution. The time-matched control values were obtained from three different experiments. In the first 15 min after exposure to aglycemic solution, the reflexes were not altered from the initial, and were not different from the control response ($P > 0.1$, Student's t test for unpaired observations) but, subsequently, there was a time-dependent depression of the reflexes ($P < 0.05$, two-way ANOVA). The cords were washed with normal physiological solution for 30 min (wash). An asterisk (*) indicates $P < 0.05$ as compared to time-matched control responses (Student's t test for unpaired observations).

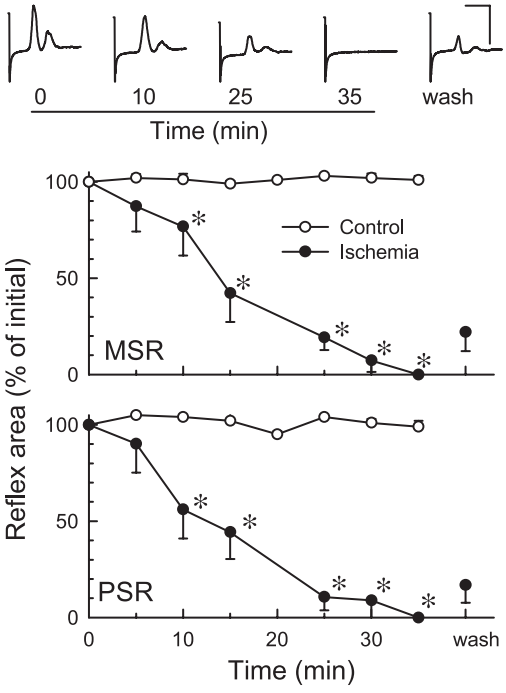

Fig. 2. Ischemia depressed the spinal reflexes. The upper panel shows the actual discharges of the reflex potentials at different time intervals after superfusion of the ischemic (glucose-free + O_2-free) solution. Vertical calibration = 1 mV and horizontal calibration = 10 ms. The lower graphs show the time-dependent depression of monosynaptic (MSR) and the polysynaptic (PSR) reflexes after exposure to ischemia. The time-matched control responses are from Fig. 1. The means ± S.E.M. values were from six different experiments. An asterisk (*) indicates $P < 0.05$ as compared to the corresponding control responses (Student's t test for unpaired observations and two-way ANOVA). The cords were washed with normal physiological solution for 30 min (wash).

Student's t test for unpaired observations). After this, there was a time-dependent depression of monosynaptic reflex and polysynaptic reflex, abolishing them within the next 15–20 min (Fig. 1). The T-50 values were about 23 min for both reflexes (Table 1). The aglycemia-induced depression could be reversed by up to 50–60% after washing with physiological solution for 30 min.

Superfusion of the ischemic solution produced a progressive decline in the magnitude of the monosynaptic reflex and polysynaptic reflex in a time-dependent manner starting from the very beginning. The reflexes were abolished within 35 min (Fig. 2). The T-50 values were between 12 and 13 min for both reflexes under ischemic condition (Table 1). The ischemia-induced effect could only be reversed by up to 10–20% after washing with physiological solution.

Superfusion of hypoxic solution decreased the magnitude of the monosynaptic reflex and polysynaptic reflex gradually from the beginning. At 10 min, the depression was about 20%, at 45 min about 35% and it remained at more or less the same level even up to 120 min. The T-50 could not be determined as the depression of reflexes was never greater than 40%. The reflexes could be reversed by more than 80% after washing with physiological solution for 30 min.

Table 1
Time required to produce 50% depression (T-50) of the monosynaptic reflex (MSR) and polysynaptic reflex (PSR) by aglycemia and ischemia after different treatments

Treatment	n	T-50 in aglycemia (min)		T-50 in ischemia (min)	
		MSR	PSR	MSR	PSR
None	6	24 ± 1.3	22 ± 1.4	13 ± 4.6	12 ± 3.5
APV (10 μM)	6	NA	Ab	64 ± 4.2[a]	Ab
CP (10 mM)	4	41 ± 3.2[a]	40 ± 2.9[a]	NT	NT
Bicuculline (1 μM)	6	NA	NA	18 ± 6.0	12 ± 7.0
Strychnine (1 μM)	6	NA	NA	32 ± 1.3[a]	31 ± 1.9[a]

The responses presented in "None" are without any antagonist and in others in the presence of agents as indicated. The means ± S.E.M. values are from n number of experiments. (a) indicates $P < 0.05$ as compared to the corresponding values in "None" (Student's t-test for unpaired observations). NA = not attained; NT = not tested; Ab = abolished; APV = DL-2-amino-5-phosphonovaleric acid; CP = creatine phosphate.

Table 2
Creatine phosphate delayed the onset of aglycemia-induced depression of the spinal monosynaptic (MSR) and polysynaptic (PSR) reflexes

Time (min)	None		Creatine phosphate (10 mM)	
	MSR	PSR	MSR	PSR
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
15	102 ± 7.0	88 ± 6.0	89 ± 0.6	93 ± 3.7
30	14 ± 14.0	5 ± 5.0	68 ± 4.8[a]	79 ± 5.9[a]
45	Ab	Ab	57 ± 9.2	43 ± 10.5

The responses (means ± S.E.M from four different experiments) are expressed as percent of initial values. (a) Indicates $P<0.05$ as compared to the corresponding values in "None" (Student's t test for unpaired observations). Ab = abolished within 33 min.

3.2. Creatine phosphate prolonged the aglycemia-induced depression

Superfusion of aglycemic solution containing creatine phosphate (10 mM) delayed the onset of depression induced by aglycemia (Table 2; $P<0.05$, Student's t test for unpaired observations). The maximum depression of reflexes observed was up to 40% for the monosynaptic reflex and 60% for the polysynaptic reflex at 45 min (Table 2) and the reflexes were totally abolished around 50 min. The T-50 values of the reflexes in the presence of creatine phosphate were around 40 min (Table 1).

3.3. NMDA receptor antagonist blocked the aglycemic and ischemic responses

Superfusion of APV (10 μM), an NMDA receptor antagonist, abolished the polysynaptic reflex and decreased the monosynaptic reflex by 32.1 ± 8.6% (see original tracings in Figs. 3 and 4 at "0" as compared to "None") which is consistent with other reports (Ohno and Warnick, 1989; Singh and Deshpande, 2002; Deshpande, 1993; Maruoka et al., 1997). In the presence of APV, the aglycemia-induced depression was not only blocked but there was a 20–60% increase in the magnitude of the monosynaptic reflex. The increase was about 60% at 30–35 min ($P<0.05$, Student's t test for paired observations) and remained at that level

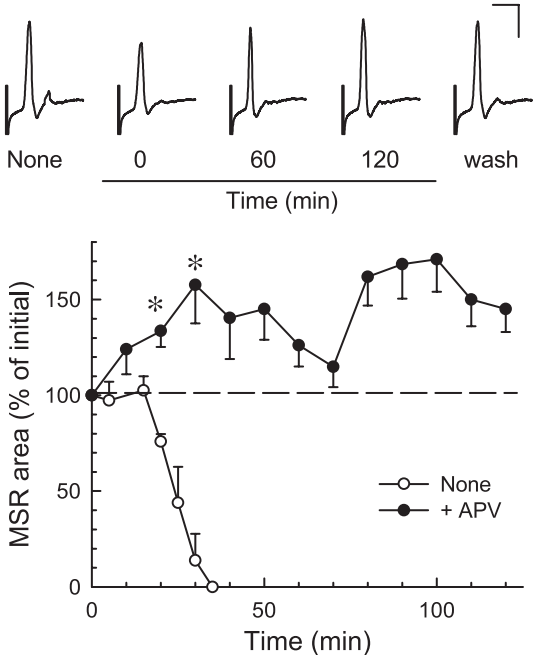

Fig. 3. The NMDA receptor antagonist blocked the aglycemia-induced depression of spinal reflexes. The upper panel shows actual reflex potentials at different time intervals after exposure to aglycemic solution in the presence of DL-2-amino-5-phosphonovaleric acid (APV; 10 μM). The reflexes at "None" and "0" are potentials before and 30 min after APV, respectively; and for "wash", after washing with physiological solution for 30 min. APV abolished the polysynaptic reflex (PSR) completely and decreased the monosynaptic reflex (MSR) by 32.1 ± 8.6%. Vertical calibration = 1 mV and horizontal calibration = 10 ms. In the lower graph, the means ± S.E.M values ($n=6$) at different time intervals after aglycemia on monosynaptic reflex in the absence (control) and in the presence of APV are presented. In the presence of APV, aglycemia could not decrease the magnitude of reflex activity while there was an increase. An asterisk (*) indicates a significant increase from "None" ($P<0.05$ Student's t test for unpaired observations). Since PSR was completely abolished in the presence of APV, no aglycemic effect on polysynaptic reflex could be estimated. The values for "None" are obtained from Fig. 1.

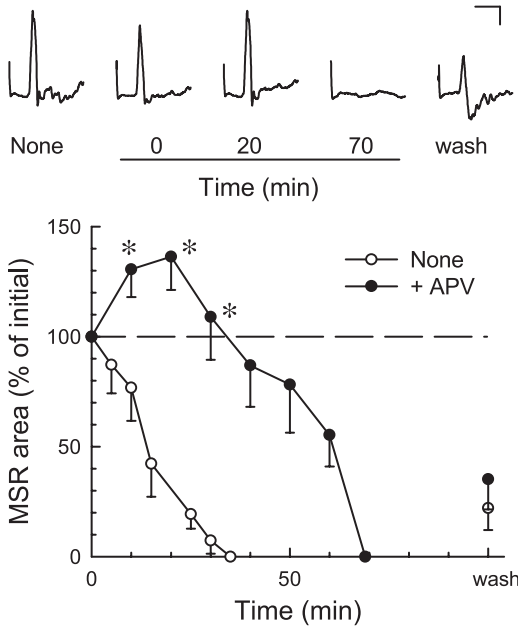

Fig. 4. The NMDA receptor antagonist blocked the ischemia-induced depression of spinal reflexes. The upper panel shows the actual discharges of the reflex potentials at different time intervals of ischemia in the presence of DL-2-amino-5-phosphonovaleric acid (APV; 10 μM). The reflexes at "None" and "0" are potentials before and 30 min after APV, respectively; and for "wash", after washing with physiological solution for 30 min. APV abolished the polysynaptic reflex (PSR) completely and decreased the monosynaptic reflex (MSR) as mentioned in legends to Fig. 3. Vertical calibration = 1 mV and horizontal calibration = 10 ms. In the lower graph the time–response values (means ± S.E.M.; $n=6$) after ischemia on monosynaptic reflex in the absence (control values obtained from Fig. 2) and in the presence of APV are presented. At the initial period up to 20 min, the reflex activity was increased as compared to "None" and indicated by * ($P<0.05$, Student's t test for unpaired observations). Subsequently there was a time-dependent depression of the reflexes, abolishing them around 70 min.

during the entire period of observation (Fig. 3). Since APV completely blocked the aglycemia-induced depression, the T-50 could not be determined.

In the presence of APV, the ischemia-induced depression was also blocked ($P<0.05$, two-way ANOVA; Fig. 4), but not completely so. There was an increase in the magnitude of the monosynaptic reflex by 40% at 20 min. Subsequently, there was a gradual time-dependent fall of reflexes abolishing them around 70 min (Fig. 4). The T-50 in the presence of APV was 64 min (five times the control; $P<0.05$ Student's t test for unpaired observations, Table 1). The monosynaptic reflex was reversed to 35% by washing with normal physiological solution for 30 min.

3.4. $GABA_A$ receptor antagonist blocked the aglycemic but not the ischemic responses

Bicuculline (1 μM), an antagonist at $GABA_A$ receptors, itself decreased the monosynaptic reflex by 7.5 ± 3.2% and increased the polysynaptic reflex by 17 ± 3.6%. In the presence of bicuculline, the aglycemia-induced depression of reflexes was completely blocked (Fig. 5). However, there was potentiation of the polysynaptic reflex activity between

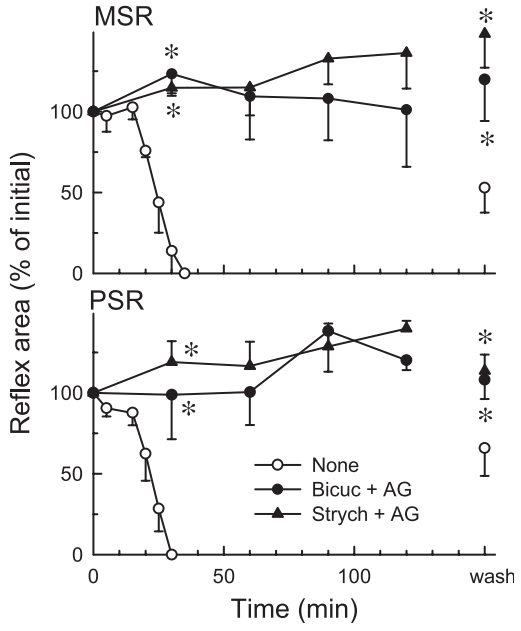

Fig. 5. Aglycemia-induced depression of spinal reflexes was blocked by bicuculline or strychnine. The graph shows the time–response relationship for aglycemia with the monosynaptic (MSR) and the polysynaptic (PSR) reflexes in the absence ("None" obtained from Fig. 1) and in the presence of bicuculline (Bicuc + AG) or strychnine (Strych + AG). After the initial recordings, the cords were exposed to bicuculline or strychnine for 30 min, then they were exposed to aglycemic medium in the presence of either. Bicuculline (1 μM) or strychnine (1 μM) blocked the aglycemia-induced depression completely ($P<0.05$, two way ANOVA). The polysynaptic reflex was increased significantly between 90 and 120 min. An asterisk (*) indicates a significant difference from "None" (Student's t test for unpaired observations). The means ± S.E.M values were from six different experiments. The cords were washed with normal physiological solution for 30 min (wash). Symbol labels are the same for both panels.

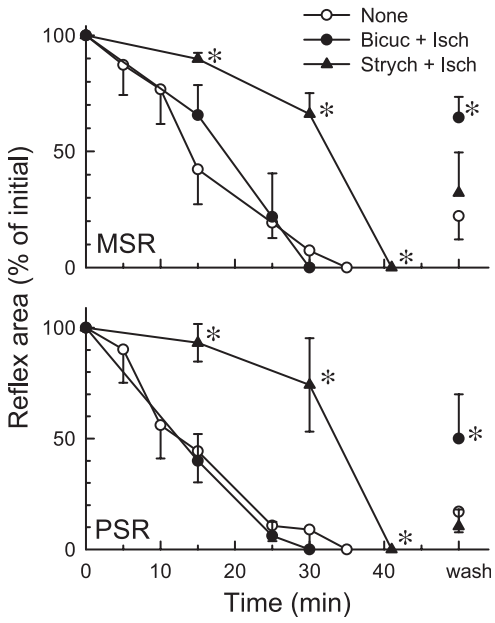

Fig. 6. Strychnine partially blocked the ischemia-induced depression of spinal reflexes but bicuculline did not. The graph shows the time–response relationship of ischemia for the monosynaptic reflex (MSR) and the polysynaptic reflex (PSR) in the absence ("None" obtained from Fig. 2) and in the presence of bicuculline (1 μM; Bicuc + Isch) or strychnine (1 μM; Strych + Isch). After the initial recordings, the cords were exposed to bicuculline or strychnine for 30 min, then to ischemic medium in the presence of either. The reflexes were depressed only up to 30% till 30 min ($P<0.05$, two-way ANOVA, as compared to "None"). Subsequently, there was an abrupt decline in the reflexes and they were abolished at 40 min. In the presence of bicuculline, the ischemia-induced response was similar to "None". The means ± S.E.M values were from six different experiments for each group. The cords were washed with normal physiological solution for 30 min (wash). Symbol labels are the same for both panels. An asterisk (*) indicates $P<0.05$ as compared to None (Student's t test for unpaired observations).

60–120 min (Fig. 5). The responses were reversed to the initial level after washing of the cord with normal physiological solution (Fig. 5). The T-50 could not be computed as there was no depression.

On the other hand, bicuculline (1 μM) failed to prevent the ischemia-induced depression of the reflexes (Fig. 6). The T-50 for the monosynaptic reflex was 18 min and for the polysynaptic reflex it was 12 min (Table 1). These values are not different from the control ($P>0.1$, Student's t test for unpaired observations). The reflexes were abolished around 30 min as observed in the control responses ($P>0.4$, Student's t test for unpaired observations). The reflexes were reversed to 50% after washing with the physiological solution.

3.5. Glycine receptor antagonist blocked the aglycemic response completely and the ischemic response partially

Strychnine (1 μM), a $glycine_A$ receptor antagonist, itself decreased the monosynaptic reflex only slightly (11 ± 6.6%) and increased the polysynaptic reflex greatly (256 ± 66.1%). In the presence of strychnine, the aglycemia-induced depres-

sion was completely blocked instead; there was an about 20–25% increase in reflex activity (Fig. 5). The T-50 could not be computed as there was no depression.

In the presence of strychnine, the time–response curve of ischemia was not similar to the control response and was shifted to the right (Fig. 6; $P<0.05$, two-way ANOVA). Even at the end of 30 min, the depression was between 25% and 30%, but after that, there was a sudden decrease in the magnitude of reflexes, abolishing them around 40 min (Fig. 6). The T-50 for both reflexes was around 30 min and they were significantly greater than the control responses (Table 1; $P<0.05$; Student's t test for unpaired observations). The reflexes were reversed by only up to 10–30% after washing with physiological solution.

4. Discussion

The present results demonstrate that ischemia and aglycemia depressed spinal synaptic transmission and abolished the reflexes concurrently involving NMDA receptors. The results further indicate GABAergic and glycinergic involvement for the aglycemia-induced depression while the ischemic response involved additional mechanisms.

In studies elsewhere, ischemia and hypoxia were produced by bubbling 95% N_2 and 5% CO_2, thus making the solution totally O_2-free (Nakai et al., 1999; Pringle et al., 1997; Luhmann and Heinemann, 1992). We performed some preliminary experiments by superfusing the deoxygenated ischemic solution produced by bubbling with 100% N_2, then we observed that the reflexes were abolished within 5 min. Therefore, we obtained a hypoxic or ischemic solution by just stopping the O_2 bubbling of the physiological solution so as to prolong or spread the time to abolish the reflexes in a step-wise manner. We were aware that the cords were still exposed to atmospheric O_2.

In the case of aglycemia but not in ischemia, the depression began after an initial resistance (Fig. 1). This resistance may have been due to the stored energy substrate available for oxidation and ATP generation. Were this the case, withdrawal of O_2 bubbling should result in no resistance and a continuous decay should follow. The instantaneous depression of the reflexes with hypoxia or ischemia supports this (Fig. 2). The supply of energy from creatine phosphate to the aglycemic medium delayed the onset of the depression induced by aglycemia (Table 2) as observed elsewhere (Luhmann and Heinemann, 1992; Deshpande et al., 1997). Therefore, continued availability of ATPs is the factor for the initial resistance in aglycemia.

If the anoxic insults involve presynaptic mechanisms, then a lessened transmitter release is expected. On the other hand, the quantity of transmitters released was greater and correlated with the degree of ATP depletion (Nakai et al., 1999; Santos et al., 1996). Thus, the depression of synaptic transmission may not have been due to the failure of presynaptic transmitter release through energy deficiency.

Membrane polarity is maintained by the energy-dependent Na^+–K^+ ATPase pump. The anoxia- or ischemia-induced depolarization can be prevented by blockers of the Na^+–K^+ ATPase pump (Stys, 1998; Santos et al., 1996), indicating the importance of the pump. Depolarization is also produced by neurotransmitters such as glutamate, 5-HT and norepinephrine (Connell and Wallis, 1989; Wang and Dun, 1990; Elliott and Wallis, 1992; Jahr and Yoshioka, 1986). These transmitters are shown to be released under ischemic conditions (Santos et al., 1996). If the membrane depolarization is due to the neurotransmitters alone, the membrane should be repolarized once the transmitter levels are reduced by washing. In our study, the reflexes did not recover fully, even after washing for 30 min. Therefore, depolarization of the membrane by neurotransmitters alone may not be the cause of synaptic depression.

Furthermore, the Na^+–K^+ ATPase pump failure stimulates reverse operation of the Na^+–Ca^{2+} exchanger system, resulting in intracellular Ca^{2+} overload (Stys, 1998, Deshpande et al., 1997; Fukuda et al., 1998), which in turn activates the Ca^{2+}-dependent enzymes such as proteases, lipases and those necessary for the generation of free radicals. These damage the membrane proteins, leading to cellular disintegration (Mattson et al., 1993; Fukuda et al., 1998). In our study, the depression may not have been due to such enzymatic effects, as the time of exposure to aglycemia or ischemia was far less than what others have reported for the cellular damage (Pringle et al., 1997; Mattson et al., 1993). The neuronal damage in these studies is seen mainly in ischemia but not in hypoglycemia. We, however, demonstrated the abolition of the synaptic reflex under both conditions. Further, if the synaptic depression is due to ATP depletion leading to $[Ca^{2+}]_i$ increase, then APV or other antagonists should not block it. Our results, on the contrary, show a blockade by antagonists (APV or bicuculline or strychnine), supporting the point that depression is mediated through the neurotransmitter-dependent mechanisms.

Glutamate and 5-HT are released during anoxic conditions (Santos et al., 1996; Pringle et al., 1997) and have been shown to depolarize the spinal motoneurons (Jahr and Yoshioka, 1986; Wallis and Wu, 1993, Long et al., 1990; Wang and Dun, 1990). The 5-HT-induced depolarization was associated with the depression of the monosynaptic reflex (Wang and Dun, 1990). NMDA also depressed the monosynaptic reflex (Singh and Deshpande, 2002). In the present study, the NMDA receptor antagonist completely blocked the aglycemia-induced depression and partially blocked the ischemia-induced depression (Figs. 3 and 4), indicating the involvement of glutamate at NMDA receptors.

Since the polysynaptic reflex is shown to be mediated via the NMDA-dependent mechanism, it was expected that aglycemia and ischemia preferentially alter the polysynaptic reflex (Maruoka et al., 1997; Singh and Deshpande, 2002). However, no such preferential depression of a polysynaptic

reflex was observed in this study (Figs. 1 and 2). Our data do not offer an explanation for this phenomenon (abolition of monosynaptic reflex/polysynaptic reflex simultaneously). Further, the lack of total protection by APV in ischemia indicates that mechanisms other than NMDA receptors are also operating in the depression of spinal reflexes.

Glutamate-induced depression of a monosynaptic reflex can also be due to the excitation of inhibitory transmission as shown elsewhere, with NMDA producing excitation of GABAergic/glycinergic inhibitory neurons (Xu et al., 2000). The release of GABA by anoxia or ischemia is reported to support the excitation of an inhibitory system by glutamate (Stys, 1998; Madl and Royer, 2000). In the present study, there was an increase in the magnitude of reflex activity in aglycemia or in ischemia in the presence of an NMDA receptor antagonist (APV). This increase may have been due to the phenomenon of withdrawal of inhibition.

Spinal cords from 5- to 6-day-old rats have yet to mature, hence the effects of aglycemia/ischemia in this preparation may not precisely reflect the actual pathophysiology of the spinal cord as seen in adult rats. However, these neonatal rat spinal cords do have GABAergic and glycinergic inhibitory systems (Deshpande and Warnick, 1988) similar to those of adult animals (Curtis et al., 1967, 1971), thus indicating functional similarity to adults. The present results demonstrate that bicuculline blocked the depression induced by aglycemia but not that produced by ischemia, while strychnine blocked the effects of both aglycemia and ischemia (though partially). The lack of effect of bicuculline on the ischemic response and the partial antagonism of the ischemic response by strychnine support the involvement of additional mechanisms for the ischemia-induced depression.

GABA and glycine coexist in the synaptic vesicles and have a common vesicular transporter for their release (Burger et al., 1991; Gasnier, 2000). They are also co-released from the presynaptic vesicles (Russier et al., 2002). Our results demonstrate that both GABAergic and glycinergic inhibitory transmissions were involved in the aglycemia-induced depression involving the mechanisms mentioned above. The depression, however, was absent in the presence of APV, indicating sensitivity of these neurons to NMDA receptors.

Depression of the reflexes by ischemia or aglycemia could also result from the excitotoxic effects on motoneurons. Thus, APV can block the depression of spinal reflexes by interfering with the excitotoxicity. Excitotoxic mechanisms have been demonstrated for the neuronal loss in the spinal cord using in vivo and in vitro experiments involving NMDA receptors (Kato et al., 1997; Regan and Choi, 1991). Thus, the depression may not have been due only to the stimulation of inhibitory GABA or glycinergic neurons but also to the exhaustion of excitatory neurons.

The involvement of neurotransmitters other than glutamate is well documented for ischemia but not for aglycemia (Santos et al., 1996; Globus et al., 1988; Nakai et al., 1999). Our findings provide evidence for the involvement of GABAergic and glycinergic systems for aglycemia also. However, it is not known why the involvement of other transmitters in hypoglycemia was not detected in the earlier study (Santos et al., 1996). This may have been due to the fact that the neurotransmitter estimations were performed on the supernatant fractions, which does not truly represent the levels of these transmitters at the synaptic cleft. Because the present experiments were performed on synaptic transmission, it was possible to detect the action of neurotransmitters efficiently. In the case of ischemia, strychnine blocked the depression only partially, but it was not blocked by bicuculline. Thus, a simple comparison between the concentrations of agonists and antagonists is not possible.

In summary, the present results indicate that aglycemia produced a degree of functional loss equal to that with ischemia. Further, our data demonstrate the involvement of glycinergic and GABAergic systems for the aglycemia-induced depression. In the case of ischemia, additional mechanisms are operative in depressing synaptic transmission. One thing is certain that an NMDA mechanism is involved under both conditions.

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