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AN *IN VITRO* MODEL OF ISCHEMIA: METABOLIC AND ELECTRICAL ALTERATIONS IN THE HIPPOCAMPAL SLICE[1]

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Abstract

The transverse guinea pig hippocampal slice preparation was used to model the metabolic changes which occur *in vivo* during ischemia and recovery. Perfusing brain slices with medium devoid of glucose and oxygen elicits rapid decreases in phosphocreatine, ATP, intracellular pH, and in the evoked field potential recorded in the dentate gyrus. AMP and creatine rise during this period, while ADP and lactate levels remain unchanged. Cyclic AMP exhibits a transient increase in concentration. With the exception of ADP and lactate, these responses are very similar to those observed during *in vivo* ischemia. The return of glucose and oxygen to the incubation medium reverses these metabolic and electrophysiological effects and also leads to pronounced elevations in cyclic nucleotide concentrations. Metabolite concentrations approach, but do not reach, *in vitro* steady state levels during the first 30 min of recovery. Total adenylate and creatine steady state levels are approximately 50% of *in vivo* concentrations. The results suggest that, although hippocampal slices differ metabolically from *in vivo* tissue, they exhibit a similar pattern of metabolic responses to ischemic and reflow conditions.

The metabolic changes which occur during ischemia *in vivo* have been detailed previously (Lowry et al., 1964; Ljunggren et al., 1974; Kobayashi et al., 1977). Metabolic changes generally appear within 30 sec of decapitation or carotid clamp ischemia; a rapid fall in phosphocreatine (PCr) and glucose levels is followed by a more gradual decline in ATP and glycogen concentrations (Lowry et al., 1964). The high rate of glucose and oxygen utilization in brain and the rapid onset of electrical failure (Hirsch et al., 1957; Urbanics et al., 1979) in all conditions which affect glucose or oxygen utilization suggest that there is a relationship between energy production (i.e., ATP) and maintenance of synaptic transmission. However, the time required for fixation of brain *in situ* has hindered accurate temporal correlations between electrophysiological deficits and metabolic changes during ischemia.

Recently, using the hippocampal slice preparation, a relationship has been shown between ATP levels and the continuation of evoked responses during anoxia (Whittingham and Lipton, 1981; Lipton and Whittingham, 1982). However, a comparison of anoxic (Kaasik et al., 1970) and ischemic (Ljunggren et al., 1974) changes suggests that ischemia produces more rapid and severe metabolic perturbations than does anoxia. Additionally, ischemia may lead to a "no-reflow phenomenon" (Ames et al., 1968) in some brain regions which could compromise recovery in those areas. If the hippocampal slice preparation can be used to model the metabolic events which occur during ischemia, specific metabolic effects on electrophysiological function during ischemic and subsequent reflow periods might be determined. In addition, the ability to manipulate the environment rapidly, to add agents to discrete regions selectively, and the absence of a blood-brain barrier combine to facilitate assessment of numerous ischemic parameters and of anti-ischemic treatments. Finally, given the flexibility of *in vitro* systems, it may eventually be possible to differentiate between the effects of oxygen and glucose deprivation and the effects due to cessation of flow.

The goals of this study were 2-fold: (*1*) to observe the metabolic changes which occur in hippocampal slices deprived of both glucose and oxygen but in the presence of continued flow, and to compare them to those changes that occur in brain following decapitation; (*2*) to determine if there are correlations between recovery of metabolite levels during an *in vitro* "reflow" period and the return of evoked potentials. Of particular interest are the levels of high energy phosphates (~P = PCr and ATP),

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intracellular pH (pH_i), and the cyclic nucleotides (cAMP and cGMP), since a perturbation in these conditions may affect synaptic transmission.

Materials and Methods

Male guinea pigs weighing 350 to 450 gm were decapitated, and their brains were rapidly removed into Krebs-Ringer bicarbonate buffer (referred to as artificial cerebral spinal fluid, ACSF) devoid of glucose and equilibrated with 95% N_2/5% CO_2 at 37°C. Standard ACSF contained 125 mM NaCl, 3.0 mM KCl, 1.4 mM KH_2PO_4, 1.3 mM $MgSO_4$, 26.0 mM $NaHCO_3$, 2.4 mM $CaCl_2$, and 4.0 mM glucose and was equilibrated with 95% O_2/5% CO_2 (pH 7.4 to 7.5). The hippocampi were removed and 400- to 500-μm slices were prepared as described previously (Lipton and Whittingham, 1979). Three experimental paradigms were used. (*1*) The excised hippocampi and subsequent slices were maintained for 7 min in 1 ml of ACSF devoid of glucose and oxygen before perfusion in the recording chamber for 33 min of recovery in standard ACSF. (*2*) Tissue was treated as in condition 1, but the initial ischemic period was extended to 15 min. (*3*) Slices were treated as in condition 1 and after the recovery period were exposed to a secondary ischemic period of 7 min by perfusion with ACSF devoid of glucose and oxygen. A secondary recovery period of 33 min followed. The three experimental paradigms are shown in Table I. Condition 3 is the *in vitro* model of ischemia and will be referred to as the "secondary" ischemia. Conditions 1 and 2 were used to represent the metabolic changes which occur following decapitation. The brain remained intact for only a portion (2 to 4 min) of the initial ischemic periods because hippocampal slices had to be prepared from the tissue during this time. During the remainder of the initial ischemia the slices were placed in 1 ml of ACSF in order to approximate *in vivo* conditions more closely. The term "*in vivo*" will be used to refer to previous studies in which the ischemic and reflow periods were examined in surgically prepared intact animals. A flow rate of 25 ml/min was maintained in the recording chamber during recovery periods and during the secondary ischemia. The term "reflow," then, refers only to the return of glucose and oxygen to the ACSF and is used for the period correlating with the return of blood flow in *in vivo* studies.

TABLE I

Experimental protocol for the evaluation of postdecapitation and in vitro ischemia in guinea pig hippocampus

Guinea pigs were decapitated and hippocampal slices were prepared as described under "Materials and Methods." *In vitro* ischemia refers to perfusion in the absence of glucose and oxygen. *In vitro* recovery refers to perfusion by ACSF containing glucose and oxygen.

Paradigm	Post-decapitation Ischemia	*In Vitro* Recovery	*In Vitro* Secondary Ischemia	*In Vitro* Secondary Recovery
	min	*min*	*min*	*min*
1	7	33		
2	15	35		
3	7	33	7	33

Decapitation — flow rate = 25 ml/min — stimulate and record

The perforant path axons of some slices were stimulated at 4 pulses/min with bipolar tungsten electrodes, and the evoked potentials were recorded in the dentate gyrus using 2- to 3-megohm tungsten electrodes. The evoked responses were amplified × 10 and displayed on a storage oscilloscope.

Individual slices were removed during the ischemic and subsequent recovery periods and frozen in liquid nitrogen. Slices were stored at −60°C. Tissue samples were homogenized in 0.3 N perchloric acid and centrifuged at 10,000 × *g* for 5 min. The supernatant was neutralized with 3 N $KHCO_3$ and stored at −60°C. The protein pellet was resuspended in 0.1 N NaOH, and protein content was determined as described by Lowry et al. (1951). PCr, ATP, ADP, and AMP were assayed using the luciferin-luciferase technique (Lust et al., 1981). Creatine (Cr) and lactate were assayed fluorometrically (Lowry and Passonneau, 1972) and the cyclic nucleotides by radioimmunoassay (Steiner et al., 1972). The sensitivity of the cyclic GMP assay was enhanced by acetylation of the cGMP (Harper and Brooker, 1975).

Adenylate energy charge (E.C.) was calculated as described by Atkinson (1968):

$$\text{E.C.} = \frac{(\text{ATP}) + 0.5\,(\text{ADP})}{(\text{ATP}) + (\text{ADP}) + (\text{AMP})}$$

Intracellular pH was estimated from the creatine kinase equilibrium (Rose, 1968), where:

$$(H^+) = \frac{(\text{ATP})(\text{Cr})}{(\text{ADP})(\text{PCr})} \times \frac{1}{K'}$$

The value used for the apparent equilibrium constant (K') was 1.41×10^8, as estimated from the data of MacMillan and Siesjo (1972). Using this K' value in our system appears to be legitimate as similar values for pH_i were obtained in normoxic slices (T. S. Whittingham and P. Lipton, unpublished data) using a different method to estimate pH_i, the 5,5-dimethyl-2,4-oxazolidinedione (DMO) weak acid technique (Waddell and Butler, 1959).

The enzymes used for metabolite analyses were obtained from Boehringer-Mannheim. Compounds used for radioimmunoassay were supplied by Becton-Dickinson. All other chemicals were obtained from Sigma. Statistical comparisons were made using the Student's two-tailed *t* test. Any differences discussed in the text were significant at $p < 0.01$ or greater.

Results

Electrophysiology. The recovery rate of the evoked response was the same following both decapitation and secondary (*in vitro*) 7-min ischemic episodes (Table II). Extending the initial decapitation ischemia from 7 to 15 min delayed the return of the evoked postsynaptic field potential (PSP) by approximately 2 min and decreased the magnitude of the maximal steady state response by 80%. The duration of the postdecapitation ischemia (7 or 15 min) did not significantly affect the electrophysiological sensitivity of the tissue to a subsequent *in vitro* ischemia. The evoked response in tissue which was previously ischemic for 15 min began to decrease at 0.75

TABLE II

Evoked response during ischemia and recovery from ischemia

In "A," the orthodromic evoked potential in the dentate granule cell layer was monitored during recovery from 7 or 15 min of postdecapitation ischemia or 7 min of *in vitro* ischemia. The perforant path was stimulated every 15 sec. The values in the "PSP" and "AP" columns are the times at which the postsynaptic field potential and the action potential portion of the evoked response first became apparent following the onset of reflow. The maximal response is the largest magnitude AP wave that could be obtained following a 1-hr *in vitro* recovery period. All values are the mean ± SEM of six determinations. In "B," the duration of the initial (postdecapitation) ischemic episode was tested for its effect on the maintenance of the evoked potential during a subsequent *in vitro* ischemic period. Slices were allowed to recover for 33 or 35 min prior to the secondary ischemia. "First decrease" is the time at which the AP portion of the evoked response first exhibits a marked (20%) decrease in magnitude. "Abolished" refers to the time at which the evoked response is isoelectic. All values are the mean ± SEM of six determinations.

A. Onset of evoked response following ischemia

Duration of Ischemia	PSP	AP	Maximal Response
min	*min*		*mV*
7	5.25 ± 0.75	9.50 ± 0.50	1.4 ± 0.3
7 (secondary)	5.50 ± 1.50	8.50 ± 2.75	
15	7.75 ± 1.00[a]	11.00 ± 1.50	0.3 ± 0.1[a]

B. Disappearance of evoked response during *in vitro* ischemia

Duration of Initial Ischemia (*In Vivo*)	First Decrease	Abolished (*in Vitro*)
min	*min*	
7	1.00 ± 0.10	1.75 ± 0.10
15	0.75 ± 0.10	1.50 ± 0.10

[a] $p < 0.01$ compared to postischemia value.

min, whereas that of tissue previously ischemic for 7 min began to decrease at 1 min of the secondary ischemia (Table IIB), although the difference was not significant. The rate of response decrement was more rapid than observed under similar conditions during anoxia in the presence of 4 mM glucose, in which the response exhibited an initial decrement at 2.25 min and was abolished in 4.25 min (Lipton and Whittingham, 1982).

Metabolites. ATP levels were minimal within 7 min of ischemia, at levels 5 to 10% of control. Extending the ischemic period to 15 min produced little additional change but did delay the recovery of ATP levels during the reflow period (Fig. 1*A*). The tissue exhibited a more rapid fall in ATP during the secondary ischemia, whereas the rate of recovery during reflow was identical whether the ischemia was a single 7-min period following decapitation or was the secondary *in vitro* 7-min period.

Concomitant with the rapid fall in ATP during the postdecapitation ischemia there was a 9-fold rise in ADP concentration, peaking at 3 min (Fig. 1*B*). No rise was observed during the secondary *in vitro* ischemia, which may be attributed to the decrease in total adenylates (see "Discussion"). AMP concentration increased in both the postdecapitation and *in vitro* ischemic slices, peaking at 7 min (Fig. 1*C*). Reflow caused a rapid fall in the AMP content of all three groups, which cannot be totally accounted for by increases in ATP and ADP during that time.

Tissue total adenylate content decreased by 50% or more within 15 min following decapitation and was independent of the onset of perfusion. No further decrement was observed during the secondary ischemic period.

Energy charge fell to minimal levels within 7 min of ischemia for both postdecapitation and *in vitro* tissue (Fig. 1*D*). The rate of energy charge recovery was the same for both postdecapitation ischemic groups (7 and 15 min) even though ATP recovery was delayed in the 15-min ischemic group. The *in vitro* model exhibited a slightly more rapid return toward steady-state levels. Steady-state energy charges following recovery from postdecapitation and *in vitro* ischemia were similar despite the decrease in total adenylate content *in vitro*.

Phosphocreatine fell more rapidly and to a greater degree than ATP during postdecapitation and secondary ischemic periods (Fig. 2*A*), with levels being minimal within 2 to 5 min. Fifteen-minute ischemic episodes delayed the recovery of PCr levels to the same extent that they delayed ATP recovery. The rates of recovery from postdecapitation and secondary ischemic periods of 7 min duration were identical. After 33 min recovery the PCr/ATP ratio was 2.34 ± 0.08, which is elevated compared to the *in vivo* control value (1.92 ± 0.05).

Creatine levels increased initially during the postdecapitation ischemia as PCr fell (Fig. 2*B*), but there was a net decrease in total creatine (PCr + Cr) from control values of 115.7 ± 4.4 nmol/mg of protein to 81.4 ± 2.8 after 7 min and 74.4 ± 2.4 after 15 min of ischemia. Total creatine levels fell slowly during the initial 30 min of recovery following 7 min of postdecapitation ischemia, stabilizing at 70.4 ± 2.4 nmol/mg of protein, 50 to 60% of *in vivo* controls. There was a greater fall in the 15-min ischemic group, to 60.2 ± 2.0 nmol/mg of protein.

The calculated pH_i, using the creatine kinase equilibrium, fell 0.4 and 0.6 pH unit within 5 min of postdecapitation and secondary ischemia, respectively (Fig. 3*A*), with an additional 0.1 unit fall when the initial ischemic episode was extended to 15 min. Upon reflow, pH_i returned to relatively stable levels within 15 min and was substantially recovered in the first 3 min. Recovery from 15-min ischemia occurred at a rate similar to that of the 7-min groups, although steady state levels were reached more slowly because of the more acidic starting point. *In vitro* steady state levels appear to be more alkaline than those *in vivo*.

Lactate levels rose 14-fold in the first 5 min and decreased between 7 and 15 min of postdecapitation ischemia (Fig. 3*B*). There was a linear decrease of lactate in both 7- and 15-min groups during the subsequent perfusion with glucose and oxygen present. Intracellular pH fell during *in vitro* ischemia despite the fact that there was no accumulation of lactate.

The changes in cAMP concentrations during ischemia and reflow were biphasic in the postdecapitation group (Fig. 4*A*). After 5 min of ischemia, there was an increase from control values of 6.64 to 200 pmol/mg of protein. After 15 to 20 min of incubation in the presence of oxygen and glucose, there was a further increase to over 600 pmol/mg of protein. The rate and degree of recovery of cAMP concentrations were slower than for the other measured metabolites. The concentration of cAMP after

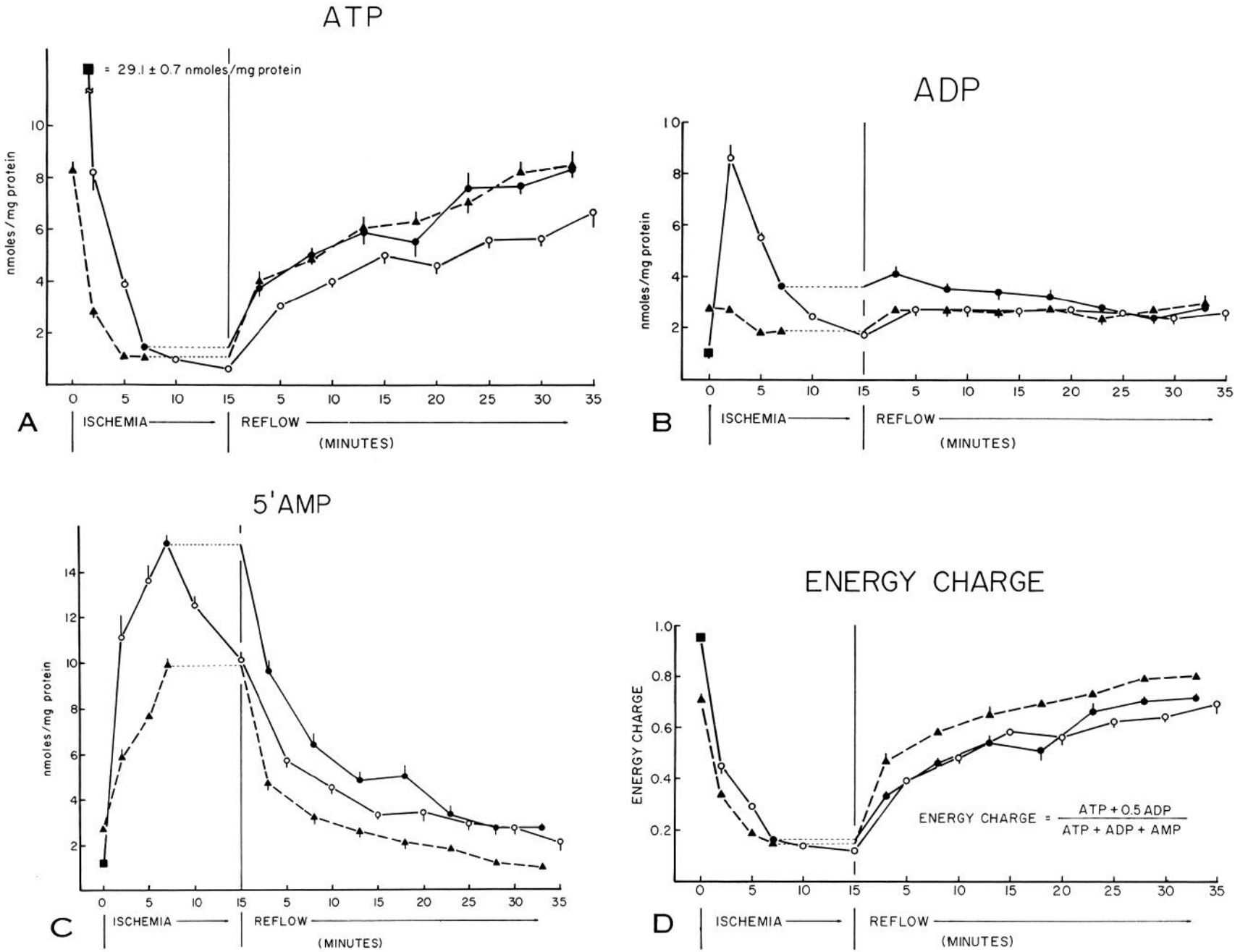

Figure 1. Changes in adenylate content and energy charge in hippocampal slices during ischemia and recovery. Treatments are as indicated under "Materials and Methods" and in Table I. ●, Paradigm 1 (7-min postdecapitation ischemia and 33-min *in vitro* recovery). ○, Paradigm 2 (as in paradigm 1, but postdecapitation ischemic period is extended to 15 min). ▲, Paradigm 3 (tissue treated as in paradigm 1 and then exposed to a secondary, *in vitro* ischemia of 7 min followed by a 33-min recovery period). ■, *In situ* value, as measured from tissue prepared by the funnel freezing technique. *A*, ATP concentration. *B*, ADP concentration. *C*, AMP concentration. *D*, Energy charge. The *in situ* concentrations of ATP, ADP, and AMP were 29.1 ± 0.7, 1.00 ± 0.19, and 1.17 ± 0.16 nmol/mg of protein, respectively. *In situ* energy charge was 0.946 ± 0.006. $N = 9$ to 13 for each experimental point; *bars* represent SEM.

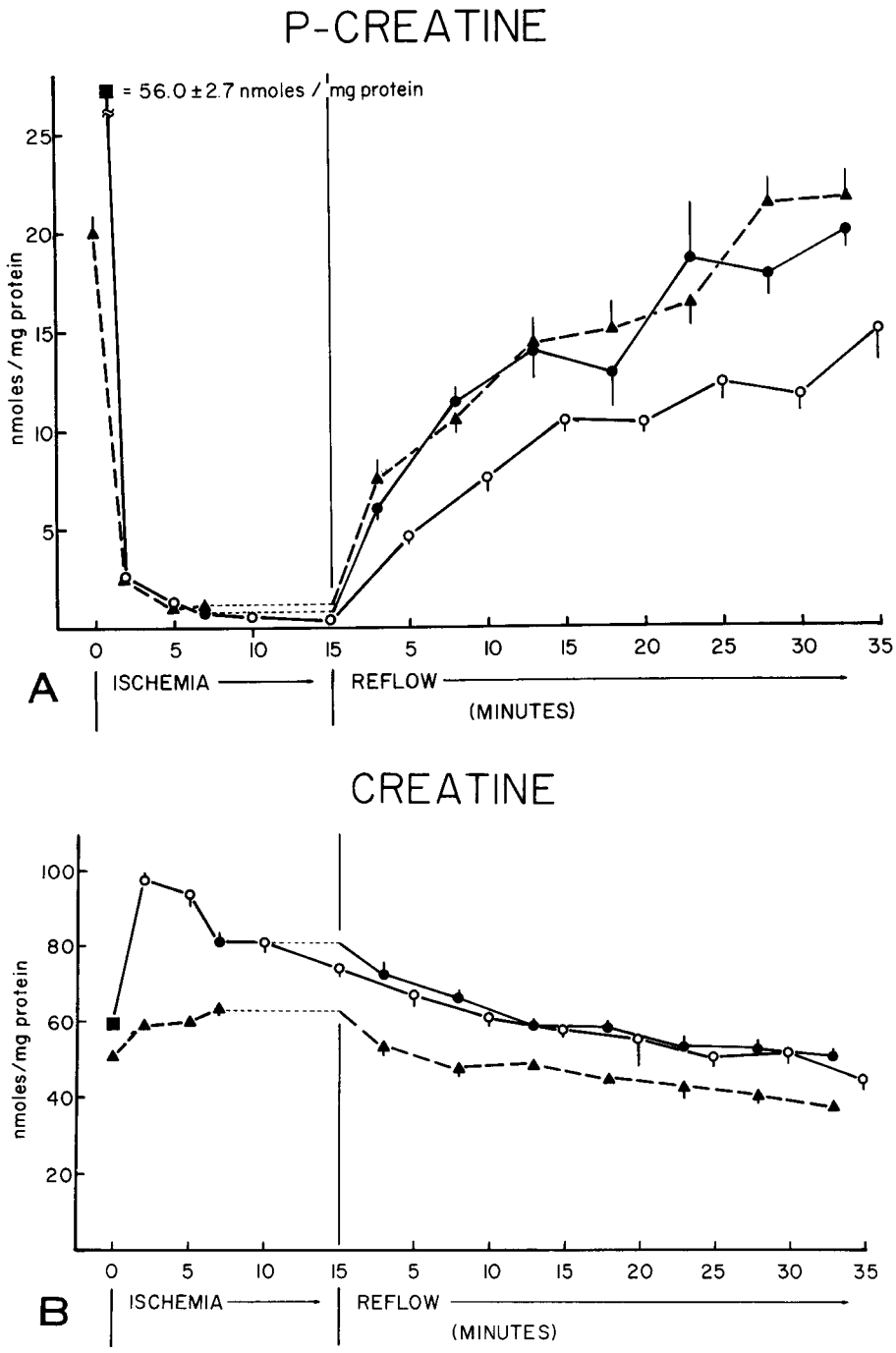

Figure 2. Changes in PCr and creatine content in hippocampal slices during ischemia and recovery. Groups are described in the legend to Figure 1. The *in situ* concentrations of PCr and Cr were 56.0 ± 2.7 and 59.7 ± 2.2 nmol/mg of protein, respectively. *A*, Phosphocreatine concentration. *B*, Creatine concentration. $N = 9$ to 13 for each experimental point; *bars* represent SEM.

7 min of ischemia and 33 min of recovery was still 70-fold greater than control values. During the secondary ischemic period, the cAMP levels fell, but there was a subsequent increase during the next recovery period similar to that in the first series.

There was no significant change in cGMP during ischemia in the postdecapitation slices (Fig. 4*B*). The increases in cGMP content in the reflow period appeared to be dependent on the duration of the initial ischemia period, as a greater increase was seen in the 15-min than in the 7-min ischemic group. During secondary ischemia, cGMP levels decreased somewhat and increased 2-fold during the subsequent reflow. As was seen with cAMP, cGMP levels returned toward basal levels more slowly than did those compounds more directly associated with energy metabolism.

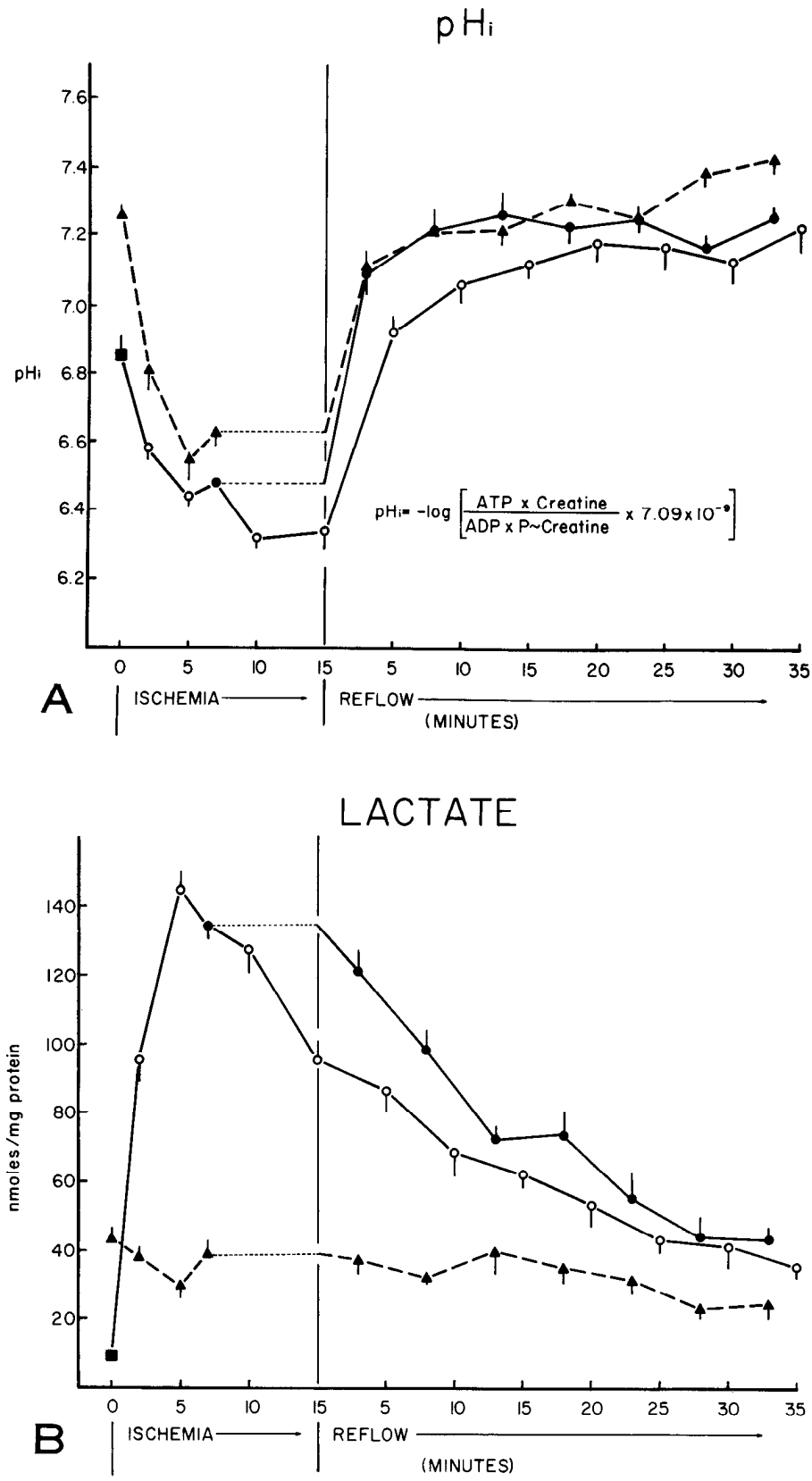

Figure 3. Changes in intracellular pH and lactate concentration from the same slices represented in Figure 1 (refer to Fig. 1 legend). Calculation of intracellular pH is discussed under "Materials and Methods." The *in situ* value of pH_i was 6.85 ± 0.06, and the lactate concentration was 8.88 ± 0.75 nmol/mg of protein. *A*, Intracellular pH (pH_i). *B*, Lactate concentration (in nanomoles per milligram of protein). $N = 9$ to 13 for each experimental point; *bars* represent SEM.

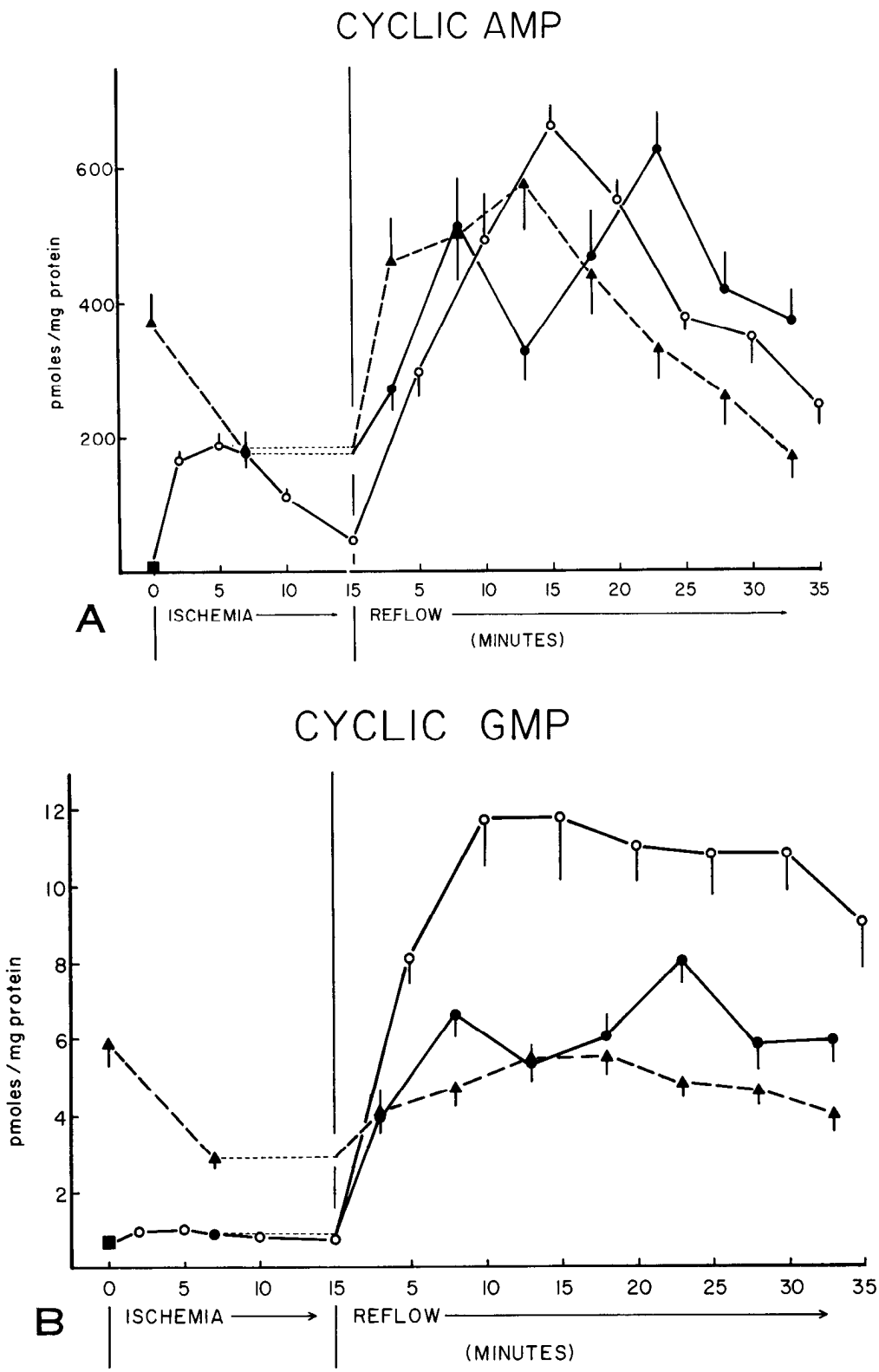

Figure 4. Changes in the cyclic nucleotide content of the slices represented in Figure 1 (refer to Fig. 1 legend). *In situ* levels of cAMP were 6.64 ± 1.59 pmol/mg of protein, and those for cGMP were 0.64 ± 0.06 pmol/mg of protein. *A*, Cyclic AMP. *B*, Cyclic GMP. $N = 9$ to 13 for each experimental point; *bars* represent SEM.

Discussion

The present study indicates that the *in vitro* hippocampal slice preparation can be used to model some of the metabolic changes which occur during *in vivo* ischemia, in respect to both rate and magnitude of change. In this study decapitation produced an ordered sequence of metabolic shifts: a rapid fall in PCr concentration and rise

in lactate and creatine levels was followed by more gradual decreases in ATP and energy charge. ADP and AMP levels rose as ATP content declined, with AMP peaking at a time when ADP concentration began to decline. These changes are in accordance with the postdecapitation observations of Lowry et al. (1964) and with a number of *in vivo* observations (Ljunggren et al., 1974; Kobayashi et al., 1977; Hansen and Nordstrom, 1979).

The *in vitro* model (secondary ischemia) produced a similar set of changes in several metabolites: PCr levels were minimal within 2 to 5 min, as was seen following decapitation. Changes in energy charge, AMP, Cr, and pH_i during the *in vitro* ischemia also resembled the changes which occurred following decapitation, although the magnitudes of the AMP and Cr peaks were decreased during the *in vitro* ischemia. The loss of the evoked response also occurred at a time similar to that required for *in vivo* ischemia and anoxia to abolish evoked potentials. For exmaple, N_2 inhalation abolished evoked potentials within 3 min in area CA 1 of the *in vivo* hippocampus of cats and rabbits (Andersen, 1960), while ischemia had a similar effect within 1 to 2 min in mammalian cortex (Williams and Grossman, 1970; Branston et al., 1974) and spinal cord (Collewijn and Van Harreveld, 1966).

However, *in vitro* ischemia produced several metabolic changes which differ from those observed *in vivo* and following decapitation. ATP levels fell more rapidly in the *in vitro* model than following decapitation. This acceleration of the ATP deficit might readily be explained by the reduced levels of ATP and PCr at the onset of the *in vitro* ischemia. In addition, the decapitation ischemia would be expected to deplete glycogen (Lowry et al., 1964) which would further reduce the slices' ability to maintain ATP levels during the early period of *in vitro* ischemia.

ADP levels did not rise during *in vitro* ischemia, as opposed to the 9-fold increase at 3 min of decapitation ischemia and the 3- to 4-fold increase reported at 1 to 2 min of *in vivo* ischemia (Ljunggren et al., 1974; Kobayashi et al., 1977; Hansen and Nordstrom, 1979). The adenylate kinase equilibrium ($[ATP][AMP]/[ADP]^2$) calculated for *in vivo* brain tissue in control and ischemic conditions has been reported to be 0.72 and 2.64, respectively (Kobayashi et al., 1977). In the hippocampal slice, the calculated equilibrium was maintained near 1.75 during ischemia and recovery. When adenylate content is high, as for the *in vivo* situation, the equilibrium would be maintained when ATP falls only if ADP concentrations increased. *In vitro* slices have a much lower total adenylate content, allowing the equilibrium to be maintained when ATP falls with only an elevation in AMP.

Lactate levels did not rise during the *in vitro* ischemia, even though pH_i exhibited a decrease similar to that following decapitation and *in vivo* ischemia (Ljunggren et al., 1974). The failure of lactate to increase during *in vitro* ischemia may result from the continued media flow during that period. This possibility is supported by the observation that lactate accumulates in postdecapitation slices maintained in a small volume of media but in the absence of flow. In addition, previous *in vitro* studies have shown that substantial lactate efflux occurs during electrical stimulation of neocortical tissue (Pull and McIlwain, 1972) and is thus available for washout.

Following decapitation, cAMP increased and cGMP remained constant, while both nucleotides increased during the recovery period. After 35 min of reflow, both cAMP and cGMP were elevated over control levels, and the concentrations of both decreased during the secondary ischemia. The *in vitro* conditions are markedly different from *in vivo*, including decreased total adenylates (and presumably guanine nucleotides) and abnormally high cyclic nucleotides at the onset of the second ischemic period.

During the recovery period there was a rise in pH_i as well as the PCr/ATP ratio. ATP, energy charge, lactate, and cyclic nucleotide levels responded more slowly to the return of glucose and oxygen. Although the initial rate of recovery was rapid, it appears that a longer recovery period is necessary to reach steady state levels *in vitro* than *in vivo*. Recovery rates were slower than those reported by Kobayashi et al. (1977) for gerbils exposed to 5 min of global ischemia. In that study, PCr levels stabilized in 5 min, as did other energy-related compounds within 30 min. The slow rate of recovery of energy compounds in brain slices has been noted previously (Rolleston and Newsholme, 1967) and is particularly pertinent to investigators using *in vitro* preparations, since slices may require recovery periods of greater than 30 min prior to data acquisition.

The 50% reduction in total adenylate and total creatine content of slices is a major consideration for metabolic studies on brain slices. The loss of these compounds occurred primarily during the postdecapitation ischemia and, in the case of the total adenylates, was greater than the 30% drop observed during 10 to 15 min of *in vivo* ischemia (Deuticke et al., 1966; Ljunggren et al., 1974). No similar loss occurred during the subsequent secondary, *in vitro* ischemia, and incubations up to 8 hr showed no further decrease. The fall in metabolite concentration was relatively independent of the duration of ischemia and suggests that the adenylate and creatine deficits may be due to tissue trauma caused by slicing or the existence of isolated compartments within individual cells.

In these same slices, values expressed as a ratio (i.e., energy charge and PCr/ATP) more closely resemble *in vivo* values than do values expressed on a per milligram of protein basis. Thus, brain slices exhibit a marked loss of some metabolites but appear to maintain the remaining portion of those metabolites in approximately the same proportions as *in vivo*. These observations suggest that either there is a loss of tissue constituents throughout the slice, or that a portion of the tissue remains viable and retains metabolites in concentrations comparable to the *in vivo* tissue. The latter explanation appears to correlate well with the metabolic profiles and physiological responses obtained from recent studies in this laboratory (Lust et al., 1982).

The second goal in this study was to identify possible metabolic changes which result in recovery of the evoked response. The present data provide no indication that ~P recovery corresponds to the return of evoked responses. There was a delay in the recovery of ATP and PCr and evoked responses in the 15-min ischemic group

compared to the 7-min group. However, the concentration of ~P was much lower in the 15-min than in the 7-min ischemic group when the evoked potential returned. It does seem possible that the reduced magnitude of the maximal response may be related to the decreased energy stores in the slices which were ischemic for longer periods (Table I). A possible explanation for the lack of correlation between ATP concentrations and the maintenance of evoked potentials may be differences in local concentration. In an earlier study, Lipton and Whittingham (1982) found that the ATP concentrations in the molecular layer (synaptic region) correlated with transmission effects more closely than concentrations in the whole slice. The concentration of ATP in discrete layers of the hippocampal slice is being investigated.

The recovery of the evoked response also occurred while cyclic nucleotide levels were greatly elevated. This suggests that whole slice cAMP and cGMP levels are not indicative of the modulatory effect on transmission that has previously been demonstrated for some cortical neurons (Stone et al., 1975). However, the effects observed by Stone et al. were on spontaneous activity, and it is quite possible that the electrical stimulation used in this study was sufficient to overcome any effect which the cyclic nucleotides might exert on normal neuronal firing patterns.

The present results also indicate that there is a more rapid loss of ATP, PCr, and the evoked response in the absence of both glucose and oxygen than in anoxia alone (Lipton and Whittingham, 1982). This suggests the importance of anaerobic glycolysis and, therefore, ATP production in maintaining synaptic transmission during the early period of anoxia.

The preceding results indicate that the *in vitro* hippocampal slice preparation can be used to model several of the metabolic changes which occur during *in situ* ischemia, particularly those associated with the adenylates and PCr. A better understanding of steady-state brain slice integrity must exist before more detailed conclusions can be extended to the *in vivo* situation. The flexibility of the *in vitro* system might then allow more accurate temporal correlations to be made between metabolic perturbations and electrophysiological deficits.

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