GABA was determined by the paper chromatographic method of Robert and Frankel as given by Colowick and Kaplan[9]. Descending chromatograms were run on whatman No. 1 paper utilizing water saturated phenol as solvent for 24 h. The dried sheets were sprayed on both sides with a 0.1% solution of ninhydrin in butanol and the colour development allowed to take place for 30 min at 90 °C. The developed spots were cut out in standards and experimental samples. Suitably chosen paper blanks were always included. The pieces of paper containing the spots were cut into small strips and eluted with 5 ml of glass distilled water. The OD of the eluate was measured in a DU2 Beckman spectrophotometer at 570 nm. Changes in glutamic and aspartic acid levels were followed by circular chromatography (on whatman No. 1 circles of 32 cm diameter), as described by Giri and Rao[10]. For quantitative estimation, the amino acid band was cut and eluted with 75% ethanol and 0.1% copper sulphate (5:1) solution. The OD of the eluate was measured in a spectrophotometer at 510 nm. The concentrations of amino acids were evaluated from standard graphs, prepared using standard glutamic and aspartic acids (BDH England). The levels of glucose in the blood (collected by cardiac puncture) were determined colorimetrically[11].

Results and discussion. It is evident from the data given in table 1 that the weight of the animal and brain exhibited considerable decrease as a function of alloxan diabetes. The blood sugar levels demonstrated 80–85% elevation as a function of disease (table 1). The level of GABA in general showed considerable decrease in the fore, mid and hind brain regions on alloxanization (table 2). It is also clear that the level of GABA exhibited regional specificity. It was more in the brain stem region and less in the mid-brain of control frogs (table 2). On inducing diabetes, the level of GABA decreased remarkably (− 72.3%) in the hindbrain, hence, this region showed the highest response during the diabetic state (table 2). It is therefore obvious that the brain stem is the region which is highly susceptible to the effects of alloxan diabetes, thus safegarding the fore- and mid-brain regions with their highly significant functional assignments.

The level of glutamic acid increased in the forebrain and decreased in the mid- and hind-brain regions on alloxanization (table 2). Following the same trend, the level of aspartic acid also exhibited an increase in the forebrain and a decrease in the mid- and hind-brain regions (table 2). The decrease in the level of GABA and a corresponding decrease in glutamic and aspartic acid levels in the mid- and hind-brain regions as a function of alloxan diabetes indicate a decrease in the production of the inhibitory transmitter in these regions. In support of this, considerable decrease in the total free amino pool of mid- and hind-brain regions was observed on inducing alloxan diabetes in frogs (unpublished observations of Nayeemunnisa, 1976). It is therefore likely that the glutamic acid conversion to GABA was inhibited by the decrease in the precursor substrate. This in turn is related to the higher respiratory rate of acute diabetic state[12] and would allow certain synaptic pathways to be facilitated and synaptic ratio to be altered so that through conduction pathways would be established[13].

The substantial decrease in the level of GABA and corresponding increase in glutamic and aspartic acid levels in the fore-brain during diabetes (table 2) indicate that the production of the inhibitory transmitter is decreased in this region probably due to substrate inhibition as the total free amino acid pool increases in the fore-brain during diabetes (unpublished observations of Nayeemunnisa, 1976). This may perhaps be related to the enhanced protein catabolism in this region during diabetes. Earlier reports of Nayeemunnisa[7] indicated that the protein metabolism is affected in the brain of frog during diabetes. It is therefore possible that an increase in protein catabolism may result as a consequence of diabetic state, which is well indicated by the increase of free amino acid levels of the fore-brain (table 2 and unpublished observations of Nayeemunnisa). Since glutamic acid and GABA are intimately involved in the CNS function, the alterations brought about in their levels by the diabetic state emphasize a correlation between the diabetic state and the altered functional dynamics of the central nervous system.

10 K. V. Giri and N. A. N. Rao, J. Ind. Inst. Sci. *34*, 95 (1952).
11 Folin and Wu, J. biol. Chem. *41*, 367 (1920).
12 M. Hennon and M. A. Dalaunay, C. r. hebd. Séanc. Acad. Sci. Paris *271*, 1420 (1970).
13 G. W. O. Oliver, P. V. Taberner, J. T. Rick and G. A. Kerkut, Comp. Biochem. Physiol. *38*, 537 (1971).

Electrical uncoupling by Sr action potentials in cardiac muscle[1]

R. Ochi

Department of Physiology, Jichi Medical School, Minamikawachi-machi, Tochigi-ken (Japan 329-04), 11 March 1977

Summary. Sr action potentials elicited in guinea-pig papillary muscle under Na-free conditions decreased the space constant and increased the input resistance. It suggests that Sr current induced intercellular uncoupling by increasing $[Sr]_i$.

The propagation of cardiac action potential requires electrical coupling between cells. The coupling is interrupted by Ca, Sr[2] and Na[3] ions electrophoretically injected into the myoplasm of Purkinje fibres as in epithelial cells[4] and by ouabain treatment in ventricular muscle[5]. I report here that similar electrical uncoupling was produced by Sr action potentials[6] which could increase $[Sr]_i$ by Sr current through the general membrane.

1 Supported by grant from Japanese Ministry of Education and from Japan Heart Foundation Research Grant for 1976 contributed by Tokyo Marine and Fire Insurance Co., Ltd.
2 W. C. De Mello, J. Physiol., Lond. *250*, 231 (1975).
3 W. C. De Mello, J. Physiol., Lond. *263*, 171 (1976).
4 B. Rose and W. R. Loewenstein, J. Membrane Biol. *28*, 87 (1976).
5 R. Weingart, J. Physiol., Lond. *264*, 341 (1977).
6 J. Vereecke and E. Carmeliet, Pflügers Arch. *322*, 60 (1971).

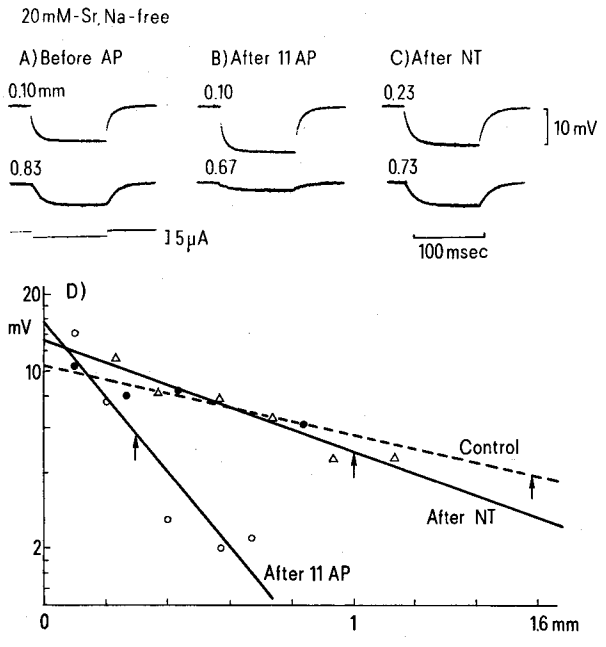

Fig. 1. Effect of Sr action potentials on electrotonic potentials of guinea-pig papillary muscle. *A–C* Specimen records of electrotonic potentials. Series *A* was performed during 6–12 min perfusion with Sr-Tyrode. Then immediately 11 Sr APs were elicited at 0.5 Hz. *B* during 9–18 min after APs. Thereafter preparation was superfused for 15 min with normal Tyrode and series *C* was done during 6–14 min after reintroduction of Sr-Tyrode. In *D*, amplitudes of electrotonic potentials were plotted against the distance between the sucrose-gap border and the penetrated points for each series. Arrows to regression lines indicate the space constants.

The membrane potentials of guinea-pig papillary muscles, cut from the right ventricle, have been recorded with glass micro-electrodes while hyperpolarizing and stimulating current pulses have been applied through a sucrose-gap[7]. The muscle was perfused by either normal Tyrode or with Na-free, Ca-free, 20 mM-Sr-Tyrode[8]. Membrane constants were obtained during Sr-Tyrode perfusion by recording electrotonic potentials at various distances[9]. After control analysis in Sr-Tyrode several Sr action potentials (APs) were elicited. They accompanied strong contractions which usually fused together to a contracture. Membrane constants were measured again with a delay of about 10 min after the stimulation.

Figure 1 shows that 11 APs reduced the electrotonic potentials recorded from the distant part of muscle and so the space constant λ ($= \sqrt{r_m/r_i}$, where r_m is the membrane resistance and r_i the internal resistance). This effect of APs was reversed by perfusing the muscle with normal Tyrode for 15 min. Figure 2 shows another experiment in which λ has been plotted. In figure 2A, the decrease of λ became apparent after 90 min perfusion with Sr-Tyrode. Then 20 APs were elicited, which made the slope of the regression line steeper with decreasing λ. These changes were almost reversible by washing the muscle with normal Tyrode. Thereafter, in figure 2B, 5 APs slightly decreased λ, while an additional 15 APs markedly decreased it. The input resistance R_0 ($= \sqrt{r_m \cdot r_i}$) is linearly related to the y-intercept of each regression line, which was increased by Sr APs in spite of the decrease of λ. It indicates that r_i was increased by Sr APs. λ was decreased in all of 9 experiments by 10 to

7 R. Ochi, J. Physiol. *263*, 139 (1976).

8 Composition of the normal Tyrode was: NaCl 142, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1, Tris-HCl 10, glucose 5 (mM); pH 7.3. That of Sr-Tyrode was: $SrCl_2$ 20, choline chloride 115, KCl 2.7, Tris-HCl 10, glucose 5 (mM); pH 7.3.

9 A. L. Hodgkin and W. A. H. Rushton, Proc. R. Soc. *B 133*, 444 (1946); S. Weidmann, J. Physiol., Lond. *118*, 348 (1952).

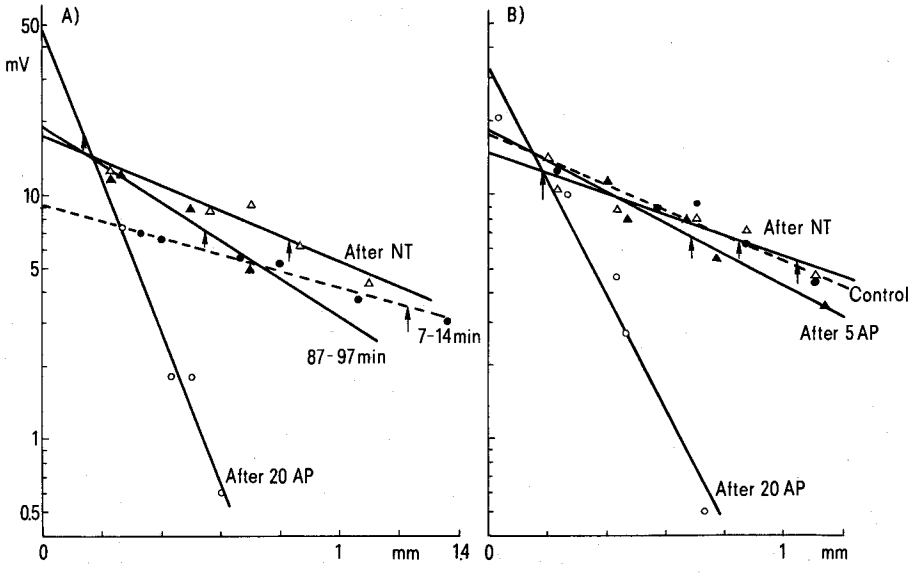

Fig. 2. Effect of Sr action potentials and long-time perfusion with Sr-Tyrode on spatial decay of electrotonic potentials. Similar plotting as in figure 1D from another experiment. 20 mM-Sr, Na-free solution. In *A*, 1st series (7–14 min ●---) and 2nd series (87–97 min ▲) were performed without applying stimulation. 3rd series (○) was done about 10 min after 20 APs. Partial recovery was obtained by normal Tyrode perfusion (4th series △). This last series is plotted as control in *B*. There 5 APs induced slight uncoupling (▲) and additional 15 APs induced marked uncoupling (○). The effect was reversible by normal Tyrode perfusion (△). Arrows indicate space constants.

23 APs from 1.31 mm (mean, range 0.81–2.00) to 0.36 mm (range 0.14–0.66). R_0 was increased in 8 out of these 9 cases, although R_0 should be underestimated because of the progress of uncoupling will increase the leakage current fraction of the applied current pulse. A tentative calculation of r_i from λ and R_0 revealed that it was increased by about 10 times by Sr APs. The increase of r_i is considered to reflect the increase of intercellular coupling resistance.

$[Na]_o$ was essential to the recovery of coupling obtained by normal Tyrode perfusion, since similar recovery was not obtained by Ca-containing, Na-free Tyrode, but by Ca-deficient, Na-containing Tyrode. The uncoupling induced by electrophoretically injected $[Na]_i$[3] and the recovery of uncoupling by $[Na]_o$ are usually explained by assuming that the Na–Ca exchange system, which transports Sr as well as Ca, participates in the regulation of $[Ca]_i$ and $[Sr]_i$[10].

The present uncoupling by Sr APs could be due to the high $[Sr]_i$ produced by large Sr current, presumably by slow uptake of Sr by intracellular stores and by decreased Sr efflux under Na-free condition. Although the coupling under normal condition is high, it might be variable depending on $[Ca]_i$ which is determined by Ca current, Ca stores and Na–Ca exchange system.

10 H. Reuter and N. Seitz, J. Physiol., Lond. *195*, 451 (1968).

Transport of sodium, water, 3-O-methyl-glucose and L-phenylalanine in vitro in biotin-deficient rats intestine

F. Petrelli, S. Coderoni, P. Moretti and M. Paparelli

Institute of General Physiology, University of Camerino (Italy), 3 March 1977

Summary. In biotin-deficient rats, a decreased intestinal transport of Na^+, H_2O and L-phenylalanine, and no transport differences of 3-O-methyl-D-glucose were observed. The lower Na^+ and L-phenylalanine transport appears to be referable to a decreased energy availability and probably not to the lack of a carrier.

It has been demonstrated that incorporation of amino acids into proteins of liver, intestinal mucosa, pancreas and skin in vivo and in vitro, is markedly decreased in biotin deficiency, and that a single injection of biotin to the biotin deficient rats stimulates amino acid incorporation, both in vivo and in vitro, more than 2fold[1–3]. The evaluation of the amino acids incorporation into liver proteins in vivo and in vitro indicated that the synthesis of some proteins was highly stimulated, while the synthesis of other proteins was not stimulated at all[2]: such a specificity has already been described[4,5]. The biotin-mediated stimulation was suppressed following treatment with inhibitors of protein or RNA synthesis, like puromycin, ethionine or actinomycin D. The effect of biotin on protein synthesis was preceded by a stimulation of the incorporation of orotic acid into nuclear and ribosomal RNA, as early as 2–4 h after biotin treatment. In the presence of nuclear RNA from biotin-treated rats, higher levels of amino acids incorporation by normal rat liver ribosomes, if compared with the incorporation in the presence of similar RNA isolated from biotin-deficient rats[1], were obtained. Further evidence suggests that the synthesis of other RNA fractions is stimulated by biotin[6–8].

Table 1. Sodium, water and L-phenylalanine transport: values obtained from everted intestinal sacs of normal control and biotin-deficient rats

Groups	Transport		
	Water ($ml/g^{-1}\ h^{-1}$)	Sodium ($\mu moles/g^{-1}\ h^{-1}$)	L-phenylalanine ($\mu moles/g^{-1}\ h^{-1}$)
Normal rats (6)	6.34 ± 1.14	911 ± 165	62.2 ± 7.5
Biotin-deficient rats (7)	3.27 ± 0.94*	470 ± 137*	43.3 ± 10.7*

Incubating fluid: Krebs-Henseleit bicarbonate (pH 7.4) + 10 mM L-phenylalanine.
Numbers in parenthesis indicate the number of animals. Values are expressed as mean + SD. * $p < 0.001$; significant difference from normals (Student's t-test).

In biotin deficiency, the energy production is impaired by decreased utilization of glucose and by decreased oxidative phosphorylation[9]; also the lipid content of mitochondria is significantly decreased[9]. The liver acetyl CoA carboxilase activity, as well as the in vivo incorporation of acetate-1-^{14}C into liver phospholipid fraction of biotin-deficient rats, was less than 50% of normal levels. The cholesterol synthesis is also altered[10]. The biotin-deficient rat liver mitochondria showed decreased phosphorylation efficiency and poor respiratory control, as compared to normal rat liver mitochondria, when NAD^+-linked substrates were oxidized. No difference between biotin-deficient and normal rat liver mitochondria in both the parameters referred to above were seen when succinate was the substrate; such results indicate that the observed loose coupling was localized at site I[11,12]. The locus of damage in energy conservation is

1 K. Dakshinamurti and S. Litvak, J. biol. Chem. *245*, 5600 (1970).
2 R. L. Boeckx and K. Dakshinamurti, Proc. Can. Fed. Biol. Soc. *15*, 200 (1972).
3 R. L. Boeckx and K. Dakshinamurti, Biochem. J. *140*, 549 (1974).
4 K. Dakshinamurti and C. Cheah-Tan, Archs Biochem. Biophys. *127*, 17 (1970).
5 K. Dakshinamurti, L. Tarrago-Litvak and H. C. Hong, Can. J. Biochem. *48*, 493 (1970).
6 F. Petrelli, G. Marsili and G. Centioni, Boll. Soc. it. Biol. sper. *49*, 1332 (1973).
7 R. L. Boeckx and K. Dakshinamurti, Biochim. biophys. Acta *383*, 282 (1975).
8 F. Petrelli, G. Marsili and P. Moretti, Biochem. exp. Biol., in press.
9 K. Dakshinamurti, V. V. Modi and S. P. Mistry, Proc. Soc. exp. Biol. Med. *127*, 396 (1968).
10 K. Dakshinamurti and P. R. Desjardins, Can. J. Biochem. *46*, 1261 (1968).
11 K. Dakshinamurti, M. A. Sabir and C. Bhuvaneswaran, Archs Biochem. Biophys. *137*, 30 (1970).
12 C. Bhuvaneswaran and K. Dakshinamurti, Archs Biochem. Biophys. *142*, 665 (1971).
13 C. Bhuvaneswaran and K. Dakshinamurti, Int. J. Biochem. *5*, 819 (1974).