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THE EFFECT OF OUABAIN ON SODIUM TRANSPORT AND METABOLISM OF THE TOAD BLADDER

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SUMMARY

10^{-4} M ouabain added to the toad bladder *in vitro* inhibited both active sodium transport and CO_2 production. These effects were similar to those observed when sodium was removed from the mucosal bathing medium. We conclude that the predominant cause of the reduction of respiration induced by ouabain is inhibition of transepithelial sodium transport.

INTRODUCTION

Since the report in 1953 by Schatzmann[1] that strophanthin prevents the uptake of potassium and elimination of sodium by erythrocytes, it has been well documented that cardiac glycosides inhibit sodium and potassium transport by a number of tissues[2-8]. Glynn[9] in 1957 presented evidence that the effect of the glycosides is on the ion transport mechanism itself rather than on its energy supply, and Skou[10] demonstrated that the effect may occur through inhibition of Na^+–K^+-activated ATPase.

Since ion transport is a major energy-requiring process of cells, it would be expected that inhibition by glycosides would secondarily reduce the rate of cellular metabolism. Inhibition of respiration by glycosides has indeed been reported in several tissues[5,6,11-14]. Although Schatzmann[1] in his original studies with erythroctyes reported no effect of cardiac glycosides on lactate production in concentrations at which ion transport was inhibited, other workers have subsequently demonstrated that lactate production is indeed inhibited[15]. Inhibition of oxygen consumption of cerebral cortex slices by ouabain (G-strophanthin) has been reported by some workers[3,16], but stimulation has been found by others[17-19]. In toad bladder, ouabain has been reported to have no effect on oxygen consumption[20,21] nor on utilization of pyruvate and acetoacetate[22], but recently inhibition of oxygen consumption of isolated epithelial cells by ouabain has been reported[23].

We are engaged in an extensive study of the effects of a number of variables on simultaneously-measured active sodium transport and CO_2 production by the toad bladder (N. S. Coplon, R. E. Steele and R. H. Maffly, unpublished.). During the

Abbreviation: s.c.c., short-circuit current.

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course of these investigations it was possible to determine the effect of ouabain on these two parameters in a variety of circumstances and thus to obtain a direct comparison of the effects of ouabain on ion transport and metabolism.

METHODS

Hemibladders from the toad *Bufo marinus* (of Colombian origin, supplied by Tarpon Zoo, Tarpon Springs, Florida) were mounted so as to separate the two halves of a glass chamber. Each side was bathed with 5 ml of a phosphate Ringer's solution (Na^+, 111; K^+, 4.0; Ca^{2+}, 1.8; Cl^-, 113; HPO_4^{2-}, 2.0; and $H_2PO_4^-$, 2.0 mequiv/l, adjusted to pH 6.5 with HCl; 220 mosmoles/kg water). CO_2-free air was bubbled through the solution at a rate of 80 ml/min. The effluent carbon dioxide was measured by a conductometric method[24], with each period of measurement being 4 min in duration. Sodium transport was continuously monitored as the short-circuit current (s.c.c.)[25] using an automatic voltage clamp. Ouabain was added to the serosal bathing medium to achieve a concentration of 10^{-4} M. Experiments were carried out both in the absence and presence of exogenous substrates, the latter (pyruvate 5 mM ± glucose 5 mM) added to the serosal bathing medium 1.5–7 h previously; malonate 5 mM was also present in some instances, but only when accompanied by pyruvate, which prevents or minimizes any inhibition by malonate of sodium transport[26,27] and metabolism (ref. 28 and N. S. Coplon, R. E. Steele and R. H. Maffly, unpublished). Results are expressed as the mean ± the standard error of the mean.

RESULTS

The effects of ouabain on s.c.c. and CO_2 production are depicted in Fig. 1. By 60 min after addition of ouabain (10^{-4} M), s.c.c. had decreased by a mean of 78 %. This is similar to the inhibition reported by others[29–31]. Concurrently CO_2 production

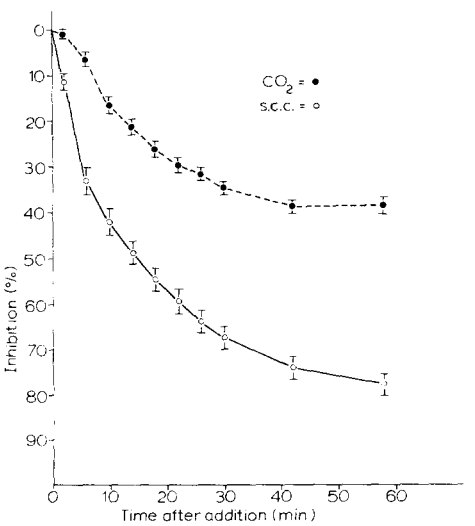

Fig. 1. Effect of ouabain on CO_2 production and short-circuit current ($n = 17$). At time 0, mean CO_2 production was 45.4 ± 3.2 μl per h (standard temperature and pressure) and mean s.c.c. was 398 ± 33 μA.

TABLE I

EFFECT OF OUABAIN IN PRESENCE OF VARIOUS COMPOUNDS AT 60 min AFTER ADDITION

Compound present	*n*	*Decrease of CO_2 (%)*	*Range*	*Decrease of s.c.c. (%)*	*Range*	*$\frac{\mu A}{\mu l/h}$*	*Range*
Pyruvate, glucose and malonate (5 mM)	4	39.5 ± 2.0	36.7–43.3	86.2 ± 4.9	75.7–99.3	20.2 ± 2.4	14.3–25.9
Pyruvate and malonate (5 mM)	8	37.0 ± 2.7	23.3–49.9	77.0 ± 3.1	60.0–89.0	18.2 ± 0.9	15.2–23.9
Pyruvate (5 mM)	3	36.9 ± 7.3	22.4–45.8	76.9 ± 3.3	70.8–81.9	14.5 ± 3.3	10.6–21.1
None	2	45.5	40.3–50.6	67.0	65.2–68.8	18.1	18.1–18.2
Total	17	38.6 ± 1.9		78.0 ± 2.3		18.0 ± 0.9	

had decreased by 39 %. The results with various compounds in the serosal bath were comparable (Table I). In a group of control experiments without ouabain ($n = 12$) in 60 min s.c.c. declined 17 % ± 2 % and CO_2 production declined 5 % ± 2 %.

To determine if the degree of reduction in CO_2 production was that expected for the degree of reduction of sodium transport, in 8 of the experiments, prior to the studies with ouabain, sodium transport was reversibly inhibited by removing sodium from the mucosal bathing medium for 60 min; this was accomplished by replacing the mucosal solution with choline Ringer's solution[32]. Sodium Ringer's was then replaced,

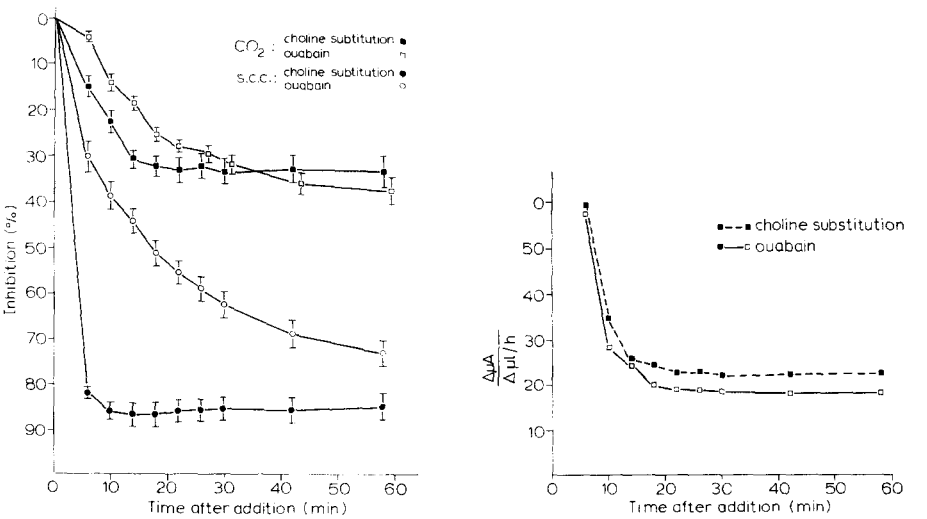

Fig. 2. Effects of ouabain and mucosal choline substitution on CO_2 production and short-circuit current ($n = 8$). At time 0, mean CO_2 production was 40.4 ± 4.9 μl/h in the choline experiments and 40.7 ± 3.7 μl/h in the ouabain experiments, and mean s.c.c. was 353 ± 51 μA in the choline experiments and 403 ± 56 μA in the ouabain experiments (paired differences not statistically significant).

Fig. 3. Ratios of the simultaneous decrements of s.c.c. and CO_2 production after ouabain versus after mucosal choline substitution ($n = 8$). Paired differences were significant ($P < 0.05$) at 26 and 30 min, and were of borderline significance ($P = 0.05$) at 42 min.

a steady-state was achieved and ouabain was added. This permitted a direct comparison of the effect of ouabain with the effect of mucosal sodium removal in the same half bladders. In Fig. 2 it is seen that substitution of choline for sodium caused a more precipitous rate of decline of the s.c.c. and CO_2 production than did ouabain. Maximum decline of the s.c.c. was reached by 14–20 min after choline substitution, whereas at 60 min the s.c.c. was still declining in response to ouabain. Nonetheless, by 60 min there was little difference between the changes induced by the two variables. When the ratio of the decrements of s.c.c. and CO_2 were compared at intervals (Fig. 3) the two curves were similar. The curve for ouabain did fall below that for choline, however, and in the periods at 26 and 30 min it was significantly lower ($P < 0.05$ by Student's "*t*" test of paired differences).

To determine if ouabain might comparably affect metabolism in the absence of Na^+ transport, ouabain was added to four half bladders where choline Ringer's served as the mucosal bathing medium. By 60 min there had been no significant change in CO_2 production ($-6\% \pm 5\%$).

DISCUSSION

Evaluation of the effects of an inhibitor of ion transport such as ouabain becomes complicated in tissues engaged primarily in transport into and out of their cells, for example cerebral cortex[33,34]. Evaluation is less ambiguous in tissues engaged in transepithelial transport, where the rate of ion transport as well as the rate of respiration can be accurately measured. In the present studies ouabain clearly reduced both the short-circuit current and CO_2 production of the toad bladder. The s.c.c. has been shown to be an accurate measure of active sodium transport by the frog skin[25] and the toad bladder[35,36]. This relationship is maintained during ouabain inhibition of sodium transport by the frog skin[37] and Herrera[31] has shown that ouabain inhibits the s.c.c. and the efflux of sodium across the serosal surface of the toad bladder to the same extent. Therefore it seems a reasonable conclusion that in our experiments ouabain reduced the rates both of active sodium transport and respiration.

Previous failure of other workers to demonstrate inhibition by ouabain of oxygen consumption of the toad bladder may have been due to simultaneous failure to produce significant depression of sodium transport[20,21]. Although it is not clear why inhibition of sodium transport was not obtained in those studies, the present method of direct measurement ensured that the degree of inhibition would be documented.

Sodium replacement with choline has previously been shown to mimic the effects of ouabain on respiration of cerebral and renal cortex slices[3,11] and of turtle and toad bladder[13,23]. However, in these studies the entire bathing medium was made sodium-free, not simply the external (mucosal) medium, and sodium removal from the internal bathing medium of the epithelial membranes might itself have affected tissue respiration. In the present experiments results similar to those with ouabain were obtained by substituting choline for sodium in the mucosal medium only. Furthermore, ouabain had no measurable effect on CO_2 production in hemibladders not transporting sodium. It is possible that the tendency of ouabain to inhibit CO_2 production to a relatively greater extent than did mucosal choline substitution reflected a further effect of ouabain on the ion transport of cells not engaged in trans-

epithelial sodium transport, and/or an effect on intracellular ionic composition, perhaps related to the loss of cellular potassium induced by ouabain[7]. However, our studies indicate that the predominant cause of the reduction of respiration induced by ouabain in the toad bladder is inhibition of transepithelial sodium transport.

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