Ca^{2+} uptake and distribution in alloxan-diabetic rat arterial and venous smooth muscle

P.D.M.V. Turlapaty, B.T. Altura and B.M. Altura[1]

State University of New York, Downstate Medical Center, Department of Physiology, Box 31, 450 Clarkson Avenue, Brooklyn (New York 11203, USA), 15 February 1980

Summary. This report demonstrates that in experimental diabetes mellitus (DM) calcium uptake and its distribution is altered in rat aortic but not in portal venous smooth muscle. Results are interpreted as consequences of increased calcium binding by aortic smooth muscle in experimental DM, which could account for the hyporeactivity of alloxan diabetic rat aorta reported previously.

Although progressive structural and functional abnormalities leading to angiopathy of macro and micro blood vessels have reported to occur in both experimental and clinical diabetes mellitus[2], relatively little attention has been devoted to calcium metabolism in these blood vessels. Several types of human diabetic blood vessels have been shown to exhibit an extensive calcification in the late stages of diabetes mellitus[3,4]. This could result in altered tone and contractility noted in these blood vessels[2].

In a recent study, we demonstrated that the reactivity of alloxan-diabetic rat aortic smooth muscle to vasoactive agents was progressively decreased, while that of venous smooth muscle remained unaltered, at least for 8 weeks[5]. Since vasoactive agents utilize different sources of cellular calcium to elicit contractile responses[6], it is possible that cellular calcium metabolism is differentially affected in these 2 types of diabetic vascular smooth muscle. However, no direct evidence is, as yet, available for the latter tenet. The present study clearly indicates that calcium uptake and distribution is altered in diabetic arterial but not in venous smooth muscle.

Methods. Male Wistar rats, weighing 350–400 g, were used in this study. 1 group of rats were made diabetic by injecting, i.p. 1 dose of alloxan monohydrate (150 mg/kg) in 0.3 ml of saline. On the same day, a 2nd group received an equal volume of saline to serve as controls. After 1, 4 and 8 weeks, the animals were sacrificed by guillotine, and blood was collected for glucose determinations. The plasma glucose concentrations (mg/100 ml) for 1-, 4- and 8-week alloxan-treated rats were 455 ± 81, 705 ± 80 and 680 ± 35, respectively. Saline-treated animals had plasma glucose concentrations of approximately 120 mg/100 ml in all 3 groups. Helically cut thoracic aortae and segments of portal veins were set up isometrically, in vitro, as described previously[7]. The vascular tissues were equilibrated for 2 h in muscle chambers aerated with 95% O_2–5% CO_2 and containing Krebs-Ringer bicarbonate solution (NKR), the composition of which has been reported previously[7]. After the initial 2-h incubation period, the tissues were exposed to ^{45}Ca containing medium (0.004 μCi/ml) for 30 min. This time period was chosen since ^{45}Ca uptake values obtained beyond 30 min, i.e., up to 120 min, were not significantly different from those obtained at 30 min[8]. At the end of this time period, each tissue was rinsed in icecold NKR for 10 sec (conventional method) or for 2 or 5 min respectively, in 50 mM La^{3+}, Ca^{2+}-free, 5 mM Tris-Ringer medium[9–12] (lanthanum method, as modified by Godfraind[13]). The 'conventional method' reveals the total exchangeable Ca^{2+} of the tissues. With the 'lanthanum method', the ^{45}Ca content is proposed to represent exchangeable membrane-bound calcium (2-min wash) and intracellular La^{3+}-resistant calcium (5-min wash)[9–12].

Results. Most of the saline control aortae exhibited spontaneous mechanical activity, similar to that previously reported[7]. On the other hand, alloxan-diabetic aortic smooth muscle preparations at 1, 4 and 8 weeks progressively lost such spontaneous mechanical activity. The amplitudes of the spontaneous contractions of alloxan-diabetic portal veins (1, 4 and 8 weeks) were significantly greater (i.e., 5, 35 and 62% over controls) than their respective saline-treated controls.

Tables 1 and 2 summarize the changes in total exchangeable, membrane-bound and intracellular calcium content of diabetic and nondiabetic rat aortae and portal veins, respectively. Since saline control rat aortae and portal veins did not exhibit any changes in calcium uptake and distribution at 1, 4 and 8 weeks, the results were pooled. Diabetic rat aortae at 8 weeks contained significantly increased membrane-bound (26% over controls) and intracellular calcium (30% over controls) content (table 1). Further, the data also revealed that calcium accumulation progressively increased in membrane and intracellular pools of aorta as the diabetes syndrome advanced (i.e., at 4 and 8 weeks). In contrast to aortae, diabetic portal veins exhibited an initial

Table 1. Total exchangeable, membrane-bound and intracellular calcium content of alloxan-diabeticc rat aorta[a]

Group	Total exchangeable calcium	2-min La^{3+}-wash calcium fraction (membrane-bound)	5-min La^{3+}-wash calcium fraction (La^{3+}-resistant)
Saline controls	4.04 ± 0.11 (16)[b]	1.84 ± 0.08 (20)	0.81 ± 0.05 (20)
Alloxan			
1 week	3.82 ± 0.14 (6)	1.77 ± 0.15 (9)	0.77 ± 0.07 (6)
4 weeks	3.82 ± 0.13 (6)	2.03 ± 0.19 (7)	0.84 ± 0.10 (6)
8 weks	4.35 ± 0.16[c,d] (6)	2.31 ± 0.18[e,c] (8)	1.07 ± 0.10[e] (7)

[a] Values are expressed in mmoles/kg wet wt (mean ± SE). [b] Number of different animals examined. [c] Significantly different from 1-week group ($p < 0.05$). [d] Significantly different from 4-week group ($p < 0.05$). [e] Significantly different from saline control ($p < 0.05$).

Table 2. Total exchangeable, membrane-bound and intracellular calcium content of alloxan-diabetic rat portal vein[a]

Group	Total exchangeable calcium	2-min La^{3+}-wash calcium fraction (membrane-bound)	5-min La^{3+}-wash calcium fraction (La^{3+}-resistant)
Saline controls	3.02 ± 0.19 (14)[b]	2.23 ± 0.12 (18)	1.61 ± 0.14 (17)
Alloxan			
1 week	3.90 ± 0.59 (6)	2.51 ± 0.28 (9)	2.36 ± 0.26[c] (9)
4 weeks	3.46 ± 0.37 (6)	2.18 ± 0.13 (6)	1.81 ± 0.36 (6)
8 weeks	2.84 ± 0.17 (5)	2.19 ± 0.42 (8)	1.68 ± 0.17 (7)

[a] Values are expressed in mmoles/kg wet wt (mean ± SE). [b] Number of different animals examined. [c] Significantly different from saline control ($p < 0.025$).

increase followed by a progressive decrease to the control level of total exchangeable, membrane-bound and intracellular calcium content (table 2). A significant increase in intra-cellular calcium content of portal vein was seen at 1 week after alloxan (table 2).

Discussion. Previously, it was reported that reactivity of alloxan diabetic rat aortae to both specific and nonspecific vasoactive agents (i.e., norepinephrine, angiotensin and potassium chloride) progressively decreased as the diabetic symptoms, reflected by sequential metabolic abnormalities, advanced[5]. Furthermore, it was also demonstrated that the fast and slow components in the contractile responses of norepinephrine, angiotensin and potassium were markedly attenuated in these alloxan diabetic rat aortic strips[5]. It is known that the initial fast components of these contractile responses utilize mainly intracellular calcium stores, whereas the maintained slow, tonic components are associated with an increased calcium influx[14]. Based on these observations, it was postulated that decreased aortic reactivity to the vasoactive agents could be due to an alteration in calcium regulation in aortic smooth muscle excised from alloxan diabetic rats that involves both sources of ionic calcium. Since the present results indicate increased calcium uptake, and increased cellular and membrane calcium content in diabetic aorta at 8 weeks, one might expect increased reactivity to vasoactive agents. However, we reported that aortic reactivity to vasoactive agents, that utilize both intra- and extracellular sources of calcium, was markedly attenuated[5]. These findings lead us to suggest that the affinity for calcium binding on membrane and intracellular sites is probably increased in arteries of the diabetic rats. This bound calcium is probably resistant to release by vasoactive agents, which could result in a lowering of the free cytoplasmic calcium available to the contractile proteins. It is unlikely that the observed changes in calcium content of aortae from diabetic rats are due to structural alterations in the membranes, since a recent study[15] demonstrated that aortic smooth muscle cells of alloxan-treated rats (8 weeks after treatment) did not reveal any structural abnormalities either at the membrane or at other subcellular sites.

In contrast to aorta, reactivity of diabetic portal veins to vasoactive agents was not modified when compared to that of the saline controls[5]. In the present study, Ca^{2+} uptake and distribution in 4 and 8 week diabetic portal veins was found to be unaltered. Since both reactivity[5] and Ca^{2+} distribution of diabetic and control portal veins were similar at 4 and 8 weeks, it is probable that the Ca^{2+} kinetics and affinity for its binding sites on the membranes and intracellular loci is not altered in alloxan diabetic venous smooth muscle of rats. As noted here in the result section, the amplitudes of the average spontaneous contractions of diabetic portal veins were greater than the saline control portal veins. One might be tempted to conclude that the initial increase in Ca^{2+} uptake and intracellular Ca^{2+}, observed after 1 week of alloxan treatment, might account for the increased resting tone of the portal veins. Such a mechanism, however, appears unlikely in view of the fact that the increases in Ca^{2+} concentration were not maintained at 4 and 8 weeks when the spontaneous activity was greatest. It is thus difficult, at the present time, to account for the progressive increase in venous tone in terms of changes in Ca^{2+} kinetics.

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Metabolism – weight relationship in some small nonpasserine birds

R. Prinzinger and I. Hänssler

Lehrstuhl Zoophysiologie, Universität Tübingen, Auf der Morgenstelle 28, D-7400 Tübingen 1 (Federal Republic of Germany), 14 February 1980

Summary. The metabolism – weight relationship 24 different nonpasserine birds follows the equation $M = 0.138\ W^{0.716}$. No pronounced differences could be found, relating to the metabolic rate per unit body weight, between these birds and representatives of the order Passeriformes.

A comprehensive analysis of the thermoregulatory process requires adequate knowledge of the levels of heat production. In most animals the basal metabolic rate varies with a fractional power of body weight. The exact value of the exponent and the complete equation for the avian basal metabolism – weight relationship appear to differ between the order Passeriformes and the other avian orders (Nonpasseres, nonpasserine birds). Previous examinations have established a higher basal metabolism of the Passeriformes. In order to examine this, we investigated the metabolism of 24 varied, relatively small (see below) representatives of the nonpasserines of 9 orders, using a standardized procedure.

Materials and methods. The bird species examined[1] are listed in the table. No previous examinations have been