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Hyperactivity of Ca Channels in Vasa Deferentia Smooth Muscle of Diabetic Rats

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SAKAI, Y. AND H. HONDA *Hyperactivity of Ca channels in vasa deferentia smooth muscle of diabetic rats.* PHARMACOL BIOCHEM BEHAV 27(2) 227–229, 1987.—Contractile responses of strips of the prostatic end of vasa deferentia isolated from streptozotocin-treated diabetic rats were investigated with five agonists. Agonists induced similar biphasic responses in vasa deferentia from diabetic and age-matched control rats six to seven weeks after streptozotocin. Twitches were observed superimposed on contractions induced by the adrenergic agonists in strips from most of the diabetic rats. Strips from the diabetic rats developed significantly greater tension than those from the controls. Especially, strips from diabetics had remarkably greater dose-related responses to Ca, and greater Ca influx, than controls when tested in the presence of either KCl or clonidine. The results suggest that both voltage-dependent Ca channels and receptor-operated Ca channels contribute to potentiation of contractile responses in diabetes.

Vasa deferentia Diabetics Hyperactivity Ca channels

DIABETES is accompanied by widespread disorders of physiological functions, but there are few studies of the functional integrity of smooth muscle in experimental diabetes. Streptozotocin-induced experimental diabetes in the rat has been shown to provide a useful model for investigation of various pathlogical signs [4, 5, 8]. Reports disagree about whether or not contractile responses of vascular smooth muscle increase in diabetes [4]. It has been reported that increased responsiveness or sensitivity of the aorta of streptozotocin diabetic rats to norepinephrine was related to the duration of the diabetic state and to extracellular calcium concentration [3]. It is widely accepted that the smooth muscle of the vas deferens, as well as vascular smooth muscle, is innervated by the autonomic nervous sytem. Denervation-induced postjunctional supersensitivity in the rat vas deferens is accompanied by increase of the maximum responses to various agonists [1]. We present here preliminary evidence of nonspecific supersensitivity of vas deferentia smooth muscle in streptozotocin diabetic rats. This evidence was obtained by examining changes in contractile responses to agonists and determining the effects on Ca mobilization.

METHOD

Diabetes was induced by a single injection of 60 mg per kg body weight of streptozotocin into the lateral tail vein of 8-week-old male Wistar rats. Age-matched male rats which were used as controls were injected with citrate solution. The rats were decapitated 6 to 7 weeks after the injections. Vasa deferentia were removed and isometric tension of the prostatic portion was recorded as described earlier by Sakai *et al.* [6]. Isometric tension was recorded on a polygraph (San-ei RECTI-HORIZ 8K). Ca influx was determined according to Meisheri *et al.* [2] by using ^{45}Ca. Diabetes was judged by the classical criterion of hyperglycemia. After the animals were decapitated, blood was collected and centrifuged at 3000 g for 10 min. Glucose was determined enzymatically with glucoseoxidase (Glucose B-Test Wako, Tokyo). The streptozotocin-induced changes were: glucose level, controls—119.8±6.7 mg/dl, diabetics—456.6±35.6 mg/dl (mean±SE), n=17; body weight, controls—474±20.1 g, diabetics—267.6±6.1 g, n=17.

RESULTS AND DISCUSSION

Figure 1 shows typical tracings of contractile responses induced in vasa deferentia from diabetic rats and age-matched controls by sympathomimetic agonists and KCl. Adrenergic drugs induced biphasic contractile responses of vasa deferentia from both diabetic rats and controls. These consisted of an initial fast (F) and a sustained slow (S) component. Spontaneous twitches extending from the F-components to the rising phase of the S-components were much more evident in the strips from the diabetics than in the controls. The most interesting feature was that both F- and S-components of responses to agonists were substantially greater in strips from diabetic rats than the corresponding responses of the strips from the controls. The F-component did not always appear in the KCl responses of either the controls or the diabetics. Increased spontaneous rhythmic activity has been observed in vascular smooth muscle. This

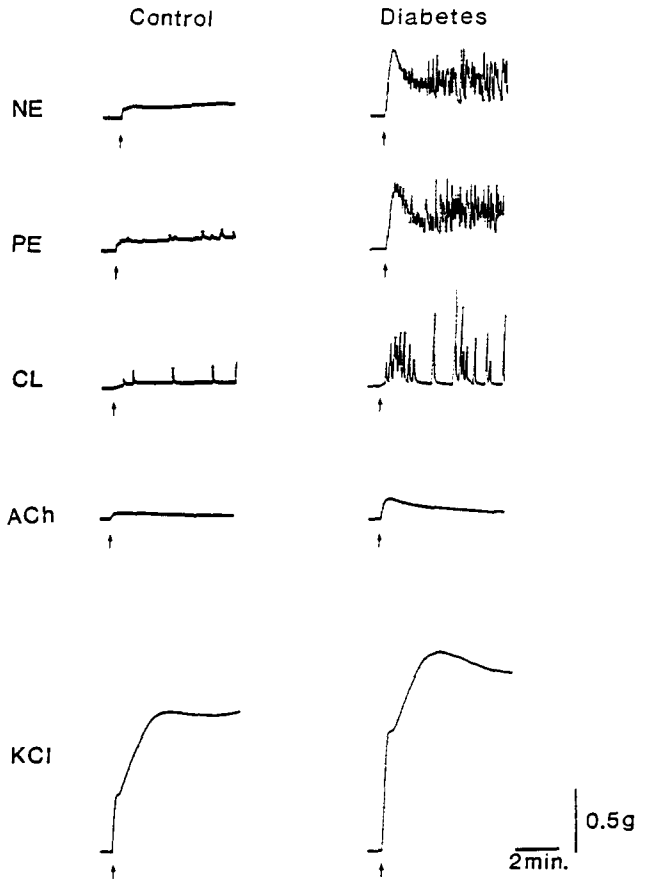

FIG. 1. Typical contractile responses of vasa deferentia to sympathomimetic drugs in diabetics and age-matched control rats. Arrow heads indicate the application of each agonist. Norepinephrine 10^{-5} M (NE), Phenylephrine 10^{-5} M (PE), Clonidine 10^{-5} M (CL), Acetylcholine 5×10^{-5} M (ACh), KCl 100 mM (KCl).

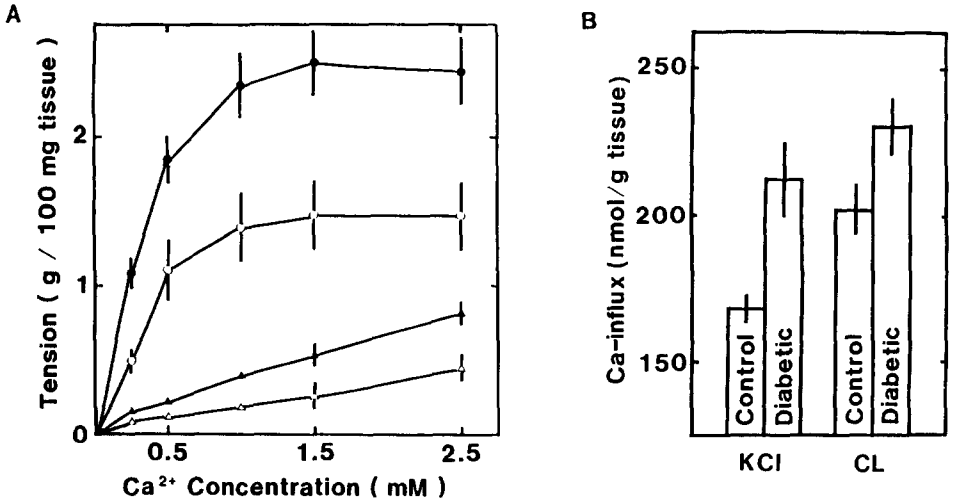

FIG. 2. (A) Dose-related contractile responses to various Ca concentrations in the presence of 60 mM KCl (●○) and 10^{-4} M clonidine (▲△) in control (open) and diabetic (closed) vasa deferentia. Values are mean±SE (n=6). (B) Ca-influx by KCl (60 mM) and clonidine (10^{-4} M) in the control and diabetic vasa deferentia smooth muscle in the presence of 1.5 mM Ca. Values are mean±SE (n=5–8).

has been attributed to syncytial communication between cells, such as that provided by gap junctions [7]. Association between rhythmic activity and formation of gap junctions in denervated rat vas deferens has been reported [9]. Autonomic dysfunction is known in many cases of diabetic neuropathy. Denervation causes supersensitivity in vasa deferentia [1]. Our results in the presence of adrenergic agonists may have been caused by such supersensitivity. Since KCl and clonidine responses were so different from each other, we studied these two agonists in more detail. Figure 2A shows dose related contractile responses to various Ca concentrations in the presence of 60 mM KCl or 10^{-4} M clonidine, and influx of Ca in the presence of KCl and of clonidine is shown in Fig. 2B. Calcium influx increased significantly ($p<0.05$) in vasa deferentia smooth muscle from diabetics in the presence of both drugs. This suggests enhanced functioning of the receptor-operated (clonidine) and voltage dependent (KCl) Ca channels in vasa deferentia smooth muscle, since potentiation of the drug effects could be due to hyperpermeability of the membrane to Ca. The increased Ca influx indicates potentiation of the KCl contractile response which is induced through voltage-dependent Ca channels, and these channels may be hyperactivated in diabetes. The increased contractile response and Ca influx in the presence of the alpha2 agonist, clonidine, may be related to changes in the number of receptors or in their efficiency. Vasa deferentia smooth muscle contractile responses mediated by postjunctional alpha2-receptors are due primarily to influx of extracellular Ca. Diabetes could alter the alpha-receptors so that they would effectively interact with clonidine to activate the channels for Ca, or other ions, or both. Increase in the population of alpha2-adrenoceptors, as a result of the disease state is possible. Our results suggest that postjunctional supersensitivity of vasa deferentia smooth muscle in diabetic rats is due to increased Ca permeability of the cell membrane.

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