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Lactate dehydrogenase activity and isoform distribution in the rat pelvic ganglion: effects of diabetes and bladder outlet obstruction

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Abstract We have previously shown that the intramural motor nerves in the rat bladder can function in anoxic conditions. The present study aims to explore the distribution and activity of lactate dehydrogenase (LDH), the key enzyme for ATP generation in anoxia. The activity and isoform distribution pattern of LDH was studied in pelvic ganglia from male and female rats. A histochemical investigation showed that the LDH activity was intense in the ganglion cells, and weak in the other tissue components (nerve bundles, connective tissue). The male pelvic ganglion weighed 55% more than the female pelvic ganglion, the enzyme activity per unit ganglion weight was 60% higher and the total LDH activity was 155% higher. The isoform distribution was similar, with M4 being dominant isoform, followed by M3H. Infravesical outlet obstruction in the female rat induced a threefold increase in ganglion weight, and the total LDH activity increased twofold. In this hypertrophic female ganglion a decreased relative amount of M4, and an increased amount of MH3, was found. Diabetes in the male rat had no effect on ganglion weight or its contents and isoform distribution of LDH.

Key words Rat · Pelvic ganglion · Lactate dehydrogenase · Isoforms · Infravesical obstruction · Diabetes

Glucose degradation is the major source of ATP regeneration in both brain [21] and peripheral [25] nerves. One reason for this is that fatty acids cannot pass the blood-brain or blood-nerve barrier [21, 25]. Most of the glucose is oxidatively degraded to CO_2 and H_2O but 10–20% will end up as lactate even under normal conditions. The ATP produced by this glycolysis is, however, only a few percent of the total. Under conditions of hypoxia, the brain lactate production can increase considerably [21] and similar findings have been reported for ischaemic peripheral nerves [24].

In a recent study on isolated rat detrusor muscle in vitro [1], we found that the amplitude of the contractile response to nerve stimulation and direct smooth muscle activation was similar in anoxia. This showed that the function of the nerve terminals was intact under these conditions. The major source of regenerated ATP in anoxia is that of glucose being degraded to lactate. When glucose metabolism is inhibited by deoxy-D-glucose, vagus nerve function was rapidly inhibited [10].

The contractile force of the detrusor muscle in diabetic rats is less sensitive than control muscle to low oxygen tension, possibly related to its higher lactate dehydrogenase (LDH) activity per milligram detrusor [2]. Little is known regarding metabolic effects of diabetes on bladder nerves or the pelvic ganglion. In the tibial nerve of the rabbit, diabetes induces a twofold increase in lactate production [8].

Infravesical outlet obstruction in the rat induces a marked hypertrophy of the bladder (see, e.g., Gabella and Uvelius [5]) as well as of the pelvic ganglion cells [6, 22]. Detrusor muscle from obstructed bladders is considerably more resistant to anoxia than control muscle, and shows a shift in the LDH isoform distribution towards the M form, which is probably less inhibited by oxygen deprivation than the H forms. As for pelvic ganglia from diabetic animals, nothing is known regarding metabolic adaptation to the growth of the ganglion cells and the increased functional demands imposed on them.

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In the present study we first carried out a histochemical investigation to determine which cells display LDH activity in the rat pelvic ganglion, and then we compared ganglia from male, female and diabetic rats and rats with infravesical outlet obstruction with regard to LDH activity and isoform distribution.

Materials and methods

Animals and surgery

Infravesical outlet obstruction. Adult female Sprague-Dawley rats weighing 220–250 g were used. The animals were anaesthetized by i.p. injection of methohexital sodium. The obstruction was induced as described previously [17]. The bladder neck and proximal urethra were visualized via a lower midline incision. A 4/0 Prolene ligature was tied around the urethra. The degree of obstruction was determined by an indwelling rod with a diameter of 1 mm. After about 50 days the animals were killed by cervical fracture, exsanguinated and the right pelvic ganglion was visualized, dissected out, weighed and immediately processed for LDH assay (for anatomy see Baljet and Drukker [3]). Control material was obtained from untreated littermates.

Diabetes. Adult male rats weighing about 250 g were given an intraperitoneal injection of streptozotocin, 65 mg kg^{-1}, dissolved in 0.1 M citrate-phosphate buffer, pH 4.2. Untreated rats served as controls. Only rats with blood glucose levels exceeding 20 mM at 2, 4 and 6 weeks after the injection were considered diabetic and included. After 10 weeks the rats were killed by cervical dislocation, bled and the right pelvic ganglion was exposed at the surface of the posterior lobe of the prostatic gland, and excised (for anatomy see Langworthy [13]). The most proximal part of the penis nerve was included in the preparation. The ganglion was then used for LDH assay as described above.

Assay of LDH activity

After weighing, the ganglia were homogenized in 100 mM phosphate buffer (pH 7.2) and centrifuged. Electrophoretic separation of LDH isoforms in the supernatant was performed on agarose gels as described previously [16]. The relative percentages of the isoforms were measured by densitometry scanning of the gels. LDH activity was determined using a commercially available photometric multiple-point rate test (Ectachem, Kodak). Activities are given in katal (1 kat corresponds to 1 mol pyruvate converted to lactate per second at 37 °C) per milligram ganglion wet weight.

Cellular localization of LDH

Pelvic ganglia were dissected out from control male and female rats and immediately transferred to isopentane in liquid nitrogen, and then put on a cryostat chuck. Sections (thickness 10 μm) were cut in a cryostat and mounted on microscope slides. Histochemical localization of LDH activity was made using the method devised by Jacobsen [11]. In brief, the mounted sections were immersed for 10 min in ice-cold acetone, followed by treatment with chloroform (−15 °C) for 15 min and, after a brief wash in acetone, dried in air. The incubation medium was made immediately before use, by a 1:1 mixture of solutions A and B. Solution A was a stock solution containing 50 mM TRIS(hydroxymethyl) aminomethane (TRIS) buffer (pH 7.2) and polyvinyl alcohol (mol. wt. about 30 kDa) 0.33 g/ml. Solution B was a fresh mixture of TRIS buffer 50 mM, sodium DL-lactate 0.4 M, nitroblue tetrazolium (Nitro BT) 9.8 mM, NaCN 10 mM and phenazine methosulfate 0.66 mM. When solutions A and B had been mixed, NAD 1.5 mM was added. The slides were incubated in the mixture for 10 min (LDH activity produces $NADH_2$, which reacts with Nitro BT, giving a dark-blue stain), then washed in warm water, fixed in 4% buffered formaldehyde, washed in acetone and mounted in glycerin-gelatin. The specificity of the staining reaction for LDH activity was ascertained by the lack of any staining if the slides had been preheated to 100 °C, if lactate had been omitted or if pyruvate (10^{-3} M) had been added.

Statistics

Values are given as mean ± SEM with the number of animals given in parenthesis. Statistical comparisons were made using the Bonferroni method of simultaneous comparisons and assuming a global probability level of 0.05 or less as indicating a significant difference.

Results

Histological localization of LDH activity

Figure 1 shows a typical section stained for LDH activity. Most of the ganglionic volume is occupied by ganglion cells. The cytoplasm of these cells is heavily stained. All profiles are stained to about the same extent. The capsule (where the dominant cells are fibroblasts) of the ganglia is weakly stained. The sections contain scattered small intraganglionic arteries and veins, and occasionally a few larger extracapsular arteries and veins. The media of these vessels show a moderate to intense staining. The tissue between the ganglionic cells, containing mainly the neuropil, and the nerves reaching and leaving the ganglia, show only weak staining.

Quantitation of LDH activity

The results from the determinations of ganglionic weight and LDH activity are presented in Table 1. The normal male ganglion weighed 50% more than the corresponding female ganglion and had a higher LDH activity, when expressed per ganglion or per milligram ganglion. The ganglia from the female rats with infravesical outlet obstruction weighed significantly more than their controls (and in fact significantly more than the control males, though not shown in Table 1). Due to the increased weight the total LDH activity had increased significantly, despite the tendency towards a lower LDH activity per milligram. There was no difference between the ganglia from diabetic male rats and their controls.

Pattern of LDH isoform distribution

The results of the electrophoretic separation of the LDH isoforms are shown in Table 2. The dominant

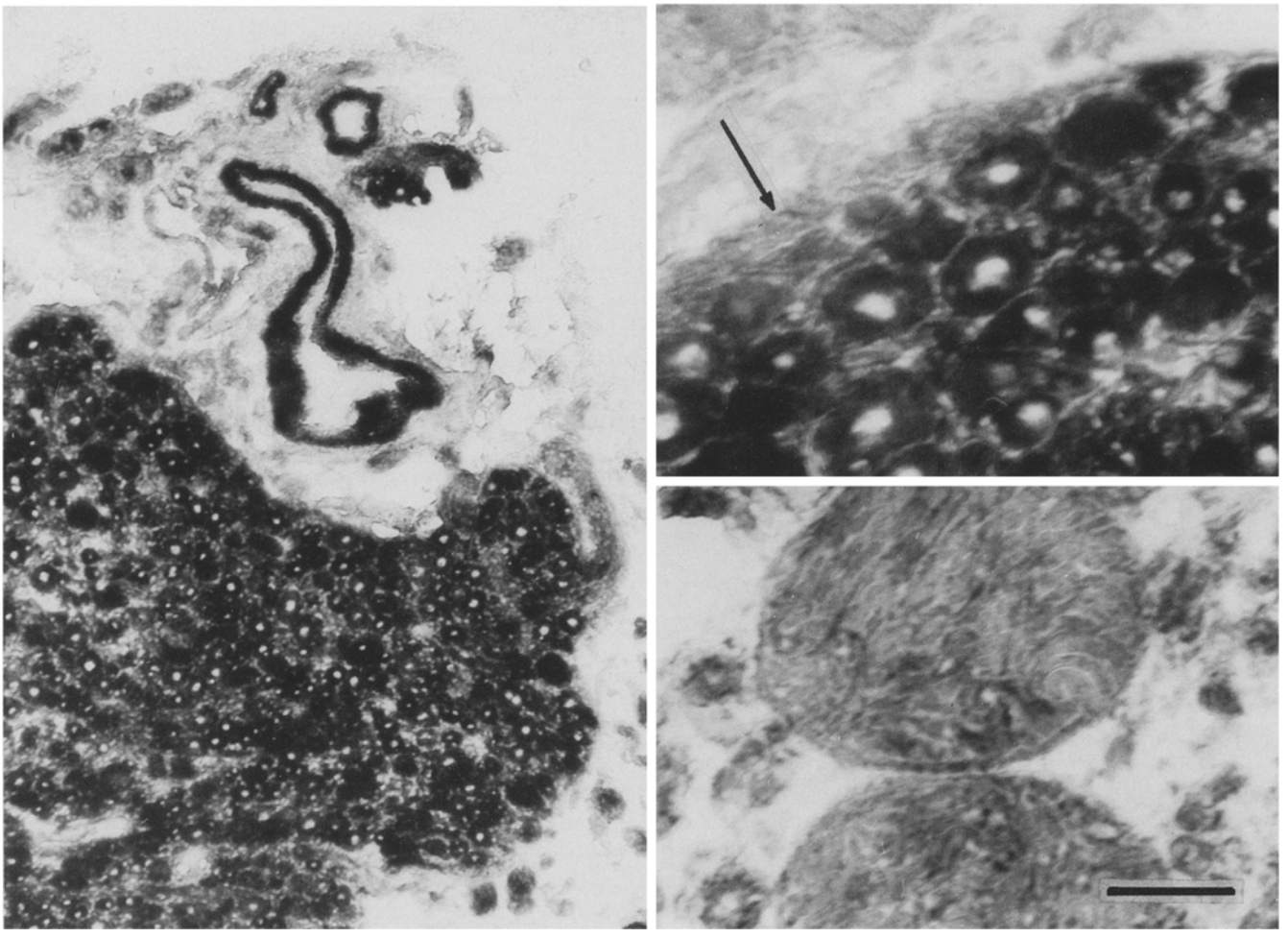

Fig. 1 Histochemical demonstration of LDH activity in a male control pelvic ganglion. *Left panel* gives an overview. The cytoplasm of the ganglion cells is intensely stained. The areas between the ganglion cells show poor staining, as does the connective tissue adjacent to the ganglion. *Arrow* indicates a blood vessel with a moderate to intense staining of the media. *Upper right panel* shows, in higher magnification, the intense staining of the ganglion cells, and the very weak staining of the ganglionic capsule (*arrow*). *Lower right panel* shows a near absence of staining in two nerves adjacent to the ganglion. *Calibration bar* corresponds to 200 μm *in the left panel* and to 50 μm in *the right panel*

isoform is M4, followed by M3H. The relative amount of MH3 was low, and H4 could not be detected. The only significant change in isoform distribution was for the ganglia obtained from the female rats with urinary outlet obstruction. In this group there was a significant decrease in the M4 isoform and a significant increase in the MH3 isoform. The relative amount of the M subunit also decreased significantly.

Discussion

Metabolic adaptations to functional disturbances have been demonstrated for the detrusor muscle of the rat bladder in diabetes [2] and infravesical outlet obstruction [1]. In diabetes, and more pronouncedly so, following outlet obstruction, an increased resistance of active force to lowered oxygen tension was demonstrated. It was also reported [1] that the contractile response to intramural nerve stimulation and to direct stimulation of the smooth muscle cells decreased to the same extent in anoxia. This shows that the intramural nerves can produce sufficient ATP in anoxia to maintain excitability, or that the stores of ATP (or phosphocreatine) are high enough to maintain nerve function despite there being no regeneration of the compounds during prolonged periods of anoxia. The latter alternative is less likely, however, as it has been shown [10] that inhibition of glucose metabolism in the vagus nerve rapidly leads to disturbances in nerve function.

If the nerve endings produce ATP in anoxia, this can only be done by glycolysis and lactate production. The conversion of pyruvate to lactate is catalysed by lactate dehydrogenase (LDH). Under normal circumstances, the ATP production from glycolysis leading to lactate formation accounts for only a few percent of the total ATP production in brain [21] and peripheral nerve [7]. The glucose ending up as lactate amounts, however,

Table 1 Weight and LDH activity of excised pelvic ganglia. Δ between groups denotes a significant difference. Statistical evaluation by the Bonferroni method of simultaneous comparison, considering a global probability level of < 0.05 as significant. Number of animals given in parenthesis (*ns* not significant)

	Female obstructed ($n = 8$)		Female control ($n = 6$)		Male control ($n = 6$)		Male diabetic ($n = 6$)
Pelvic ganglionic weight (mg)	3.72 ± 0.49	Δ	1.24 ± 0.11	Δ	1.92 ± 0.17	ns	2.19 ± 0.09
LDH activity (nkat/mg)	1.52 ± 0.15	ns	2.11 ± 0.16	Δ	3.44 ± 0.18	ns	3.02 ± 0.19
Total LDH activity (nkat)	5.24 ± 0.32	Δ	2.55 ± 0.16	Δ	6.51 ± 0.41	ns	6.56 ± 0.32

Table 2 Lactate dehydrogenase isoform distribution in excised pelvic ganglia expressed as percentage of total activity. * Significantly different from corresponding control group, $^{\Delta}$ significantly different from all other groups. Statistical evaluation by the Bonferroni method of simultaneous comparison, considering a global probability level of < 0.05 as significant. Number of animals given in parenthesis

	M4	M3H	M2H2	M1H3	H4	M/(M + H)
Male controls (6)	44.6 ± 2.6	33.3 ± 1.4	16.3 ± 0.9	5.7 ± 0.6	0	79.2 ± 1.2
Male diabetic (6)	45.7 ± 2.1	33.0 ± 0.6	16.0 ± 0.9	5.3 ± 0.6	0	79.8 ± 1.1
Female controls (6)	48.6 ± 1.0	33.1 ± 0.3	14.1 ± 0.5	4.3 ± 0.5	0	81.5 ± 0.6
Female obstructed (8)	39.4 ± 1.5*	31.8 ± 0.8	18.7 ± 1.0	$10.1 \pm 1.2^{\Delta}$	0	76.0 ± 0.7*

to about 20% of the amount of glucose metabolized [7] (due to the blood-nerve barrier only glucose can be metabolized). It is possible that the ATP produced by glycolysis is used preferentially for some cellular processes (cf. glycolysis in vascular smooth muscle which has been suggested to produce the ATP utilized by membrane pumps [19]). In hypoxia or ischaemia the nerve lactate production can increase considerably [21, 24]. It is impossible to separate the metabolism of the intramural nerves (which constitute a very small fraction of the bladder wall volume) from that of the muscle.

In the present study we have shown that most of the LDH activity in the pelvic ganglia is confined to the nerve cells, and that these cells have a high LDH activity. Judging from the intensity of the histochemical reaction, the LDH activity in the ganglion cells is level with or higher than that of the blood vessels found in the specimens. Blood vessels in general [18] have a high glycolytic activity and lactate production, which makes them capable of producing ATP to maintain part of the contractile activity in anoxia. The finding [1] that the bladder nerves are excitable in anoxia suggests that lactate production, at least in these distal parts of the nerve cells, can produce enough ATP to maintain cell homeostasis. The distribution of LDH isoforms in the ganglia with the dominance of M subunits is similar to that found in the bladder muscle cells [18, 20] and is such that the LDH activity should be maintained in hypoxia, making it possible for lactate to accumulate.

The pelvic ganglion in the male rat contains almost 3 times as many ganglion cells as that in the female [9]. The increased weight of the ganglion in the present study reflects this. It is not surprising that the total LDH activity is higher; what is measured is something that indirectly reflects the number of ganglion cells. The lower LDH activity per unit weight in the female ganglion is interesting and could suggest a lower lactate-producing capacity of its ganglion cells, but might also be explained by, e.g. a different relationship between ganglion cells and capsule tissue (in a small ganglion the surface-to-volume ratio will be higher).

Diabetes has been reported [4] to change the LDH isoform distribution pattern in hamster liver cells which are dependent on insulin for their glucose uptake. No effect of diabetes on the isoform pattern was found in the rat detrusor muscle [2], in which glucose uptake was not affected by insulin. An increased LDH activity per unit detrusor muscle volume was, however, found. In peripheral nerve bundles (which are also independent of insulin for glucose uptake) in the rabbit, diabetes has been reported to induce an increase in lactate formation [8], possibly induced by "endoneurial hypoxia" secondary to changes in the blood supply of the nerves [14]. In the rat, diabetes also leads to hypertrophy [23] of the ganglion cells projecting to the bladder, but this is probably secondary to the increased functional demands (increased diuresis) of the bladder in diabetes. In the complex pelvic ganglion of the male rate, the relative number of ganglion cells projecting to the bladder is limited [12]. The absence of any weight increase in diabetes for the male ganglion in the present study is in accordance with this. Our results show that diabetes has no effect on LDH activity or isoform pattern of the male pelvic ganglion cells in general.

Infravesical outlet obstruction leads in the female rat to pronounced hypertrophy of the detrusor smooth muscle cells [5]. In such muscle a pronounced resistance of the contractile force to hypoxia and anoxia is found [1] simultaneously with a shift [16, 20] in LDH towards more M and less H subunits. The detrusor muscle in obstruction is exposed to a considerable increase in functional load with an increased micturition pressure and a prolonged micturition [15]. The outlet obstruction also induces a dramatic increase [6, 23] in size of the nerve cells in the pelvic ganglia. This indicates a relationship between the size and functional load on the target organ, and the size of the ganglion cells. In the present study we found that the weight of the pelvic ganglion increased threefold. We had expected that possible changes in isoform distribution would be in the same direction as in the bladder muscle. The result was the opposite; the relative decrease in M4 isoform and the increase in MH3 isoform is contrary to what would be expected if the ganglion cells had adapted to hypoxia. Note also in this context the clear trend towards a decreased LDH activity per milligram in the ganglia from the obstructed rats. At present we do not know if the results should be interpreted as suggesting that the hypertrophic ganglion is less dependent in vivo on glycolysis leading to lactate formation.

The present study has described effects of sex, diabetes and infravesical obstruction on LDH activity per milligram ganglion and on the isoform distribution. The measured enzyme activities in homogenized ganglia have to be considered as representing the maximum activity in vivo (cf. ref. [16]). The level of LDH activity in intact pelvic ganglia is not known. We are planning to measure oxygen consumption and lactate production in isolated ganglia with the method we have previously used on bladder muscle [1, 2]. It is, however, difficult to find a parameter that will show in a simple manner the functional state of the ganglionic cells.

In conclusion. Most of the LDH activity in the pelvic ganglia is confined to the nerve cells. The male pelvic ganglion contains more LDH than that in the female, mostly reflecting its bigger size. Diabetes has no effect on LDH activity or isoform pattern. There is a shift from M to H units of LDH in the pelvic ganglion following infravesical bladder obstruction, contrary to what could be expected from studies of the detrusor smooth muscle under the same conditions.

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