The Acute Effect of Insulin on Capillary Endothelial Cells

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SUMMARY

A quantitative morphologic study of the capillary endothelium of striated muscle was performed in diabetic rats one hour after injection of insulin. Diabetic and nondiabetic rats injected with saline constituted the reference groups. In a randomized sample of capillary profiles, morphometric data on micropinocytotic vesicles (MPV) and on various capillary characteristics were collected.

The numeric density of MPV was higher in diabetic rats injected with insulin than in diabetic rats injected with saline (44.2 ± 8.5 versus 34.0 ± 4.5 MPV/μ^2 endothelial cytoplasm (mean ± S.D.), 2p = 0.045), thereby approaching the control value of 48.0 ± 7.3, 2p = 0.51. The insulin-injected animals, furthermore, differed markedly from the diabetic reference group as regards the ratio of free-to-attached vesicles, since they showed a large increase in the numeric density of free MPV (1.40 ± 0.18 versus 1.00 ± 0.23/μ circumference, 2p = 0.015, control value 1.59 ± 0.20) and a small decrease in the density of attached MPV (0.19 ± 0.03 versus 0.23 ± 0.02/μ circumference, 2p = 0.022; controls: 0.15 ± 0.04). Both the height of the endothelial cytoplasm and the size of the MPV were similar in all groups.

These findings may be the morphologic counterpart of the clinically demonstrated decrease in plasma volume and in the intravascular pool of albumin in diabetic patients after insulin injection. The present results demonstrate that insulin has an acute effect on the endothelial cells, including a change in the kinetics of the vesicular transport pathway. DIABETES *27*:745-49, July, 1978.

The main action of insulin is believed to be its effect on intermediary metabolism. It is also well known that insulin causes hyperpolarization of certain biologic membranes.[1] The relationship between the action on metabolism and that on ion fluxes is still not known. Reports have appeared suggesting yet another type of insulin action, namely, on the cardiovascular system, which may or may not be related to the two types of action mentioned above. Injection of insulin results in a fall of arterial blood pressure in patients with autonomic neuropathy[2-4] but not in normal subjects or in diabetics without neuropathy.[5] However, plasma norepinephrine is increased 30 to 45 minutes after intravenous injection of insulin in diabetics without neuropathy even when blood glucose concentration is not low.[5-7]

It has recently been shown that intravenous administration of insulin in such patients results in a decrease in plasma volume and a reduction in the intravascular pool of albumin.[5] The evidence indicated, therefore, that the hypovolemia caused by insulin is counterbalanced by an intact adrenergic nervous system, maintaining arterial blood pressure at normal levels at the expense of an increase in heart rate[5,8] and a decrease in peripheral blood flow.[5] The changes are not due to hypoglycemia and they are more pronounced in the feet-down, tilted position.[3-5]

The mechanism of the hypovolemic effect of insulin is hitherto unexplained, but the enhanced disappearance of albumin from the vascular space points to an increase in capillary permeability for large molecules. The passage of large molecules across the endothelial cell takes place, in all probability, by vesicular transport.[9] We report here a low density of endothelial micropinocytotic vesicles in muscle capillaries from diabetic rats and a near normalization of this abnormality one hour after insulin administration.

MATERIAL AND PROCEDURE

Female Wistar rats weighing about 200 gm. were used for the experiment. Diabetes was induced by intravenous injection of streptozotocin (65 mg. per

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kilogram body weight), and the animals were left without treatment for one week. At the time of the experiment, nonfasting blood glucose value in the diabetic rats was 473 ± 107 mg./100 ml. (mean ± S.D.) with a range of from 360 to 720 mg./100 ml. Five diabetic rats were given insulin intramuscularly (4 I.U. Actrapid, Novo), and five diabetic rats and four control rats were given saline injections. When blood glucose concentration, as monitored by Dextrostix (Ames), had fallen to about 200 mg./100 ml. (subsequently determined with the glucose oxidase method: 232 ± 20 mg./100 ml.) in insulin-injected rats, the animals were anesthetized by intraperitoneal diazepam (10 mg. per kilogram) and mebumal (15 mg. per kilogram), and a hindlimb muscle biopsy was cut out with a scalpel. The time-lag between the insulin or saline injection and the biopsy procedure was 55 to 85 minutes.

METHODS

Electron Microscopy

Small blocks of tissue were fixed in 1 per cent OsO_4 in veronal buffer for one hour, and after storage in the buffer for 18 hours at 4° C. they were dehydrated and embedded in Vestopal. Thin sections (gray to dark gray interference color corresponding to a thickness of 40 to 50 nm.) were cut on an LKB Ultrotome. Electron micrographs were obtained at a final magnification of 53,000.

Random sampling of capillaries was performed in the following way: each section was mapped at a lower magnification, and all complete vascular profiles were numbered. An average of 17 vascular profiles was selected from two to four blocks per animal using a random-number table. The complete wall was photographed in all these profiles; if more than one exposure was necessary to cover the profile, montages of the micrographs were made subsequently. All vascular profiles in which pericytes constituted more than 33 per cent of the outer boundary of the wall were considered possible larger vessels and were discarded; all cells in the vascular wall without visible contact with the lumen were defined as pericytes. An average of 14 capillaries per rat was used for the morphometric analysis.

The endothelium was divided into three segments (see figure 1): (1) the nuclear zone, limited by two straight lines tangential to the nuclear profile; (2) the junctional zone, the area on each side of the junction within which no vesicles are present, limited by two straight lines tangential to the nearest vesicles. In some cases, when vesicles were seen close to or attached to the junction, the latter was part of one of the limits;(3) the peripheral zone constituted the remaining segment of the endothelium. Vesicles were counted as "attached" when continuity between the membrane of the endothelial cell and that of the vesicle was observed. Vesicles without contact with the cell membrane in the given section were denoted "free."

Morphometry

A square grid (see figure 1), reproduced photographically on a Kodak Microlith type 3 film, was superposed on the micrographs in a random position. Its coarse lattice was used to determine the fractional volume of capillary lumen, endothelial nuclei and cytoplasm, and the surface density of endothelial cell membranes as well as the fractional length of the capillary wall covered by pericytes. Every fourth square was used for the unbiased determination[10] of the numeric density of free vesicles. The fine lattices within these squares were used to determine the fractional volume of vesicles and the surface density of vesicle membranes without distinguishing between free and attached ones. The attached vesicles were counted along the total length of the luminal and abluminal cell membrane. All other quantities were calculated from the above primary estimates.[11] All ratio estimators were calculated from the summation in the respective numerators and denominators over all capillaries in each rat.

The section thickness is relatively large compared with the vesicular dimensions, and this leads to an overestimation of the absolute numeric density of the vesicles (the so-called Holmes effect[11]). However, since there were no differences in vesicular size (table 1) the relationship between the values in the three groups is unaffected.

Since only vesicles cut through the stalk are identifiable as attached ones, the true number of such vesicles is underestimated, and the difference (true minus apparent) is included in the number of free vesicles.[12] Therefore the ratio free/attached vesicles is overestimated. This bias is more accentuated the smaller the ratio is.

In each animal about 260 square points of the grid were counted, corresponding to an area of 60 μ^2 of endothelial cytoplasm. All measurements were performed blindly on coded micrographs by one technician.

The statistical significance of differences between mean values was evaluated at a 5 per cent limit using student's unpaired t-test.

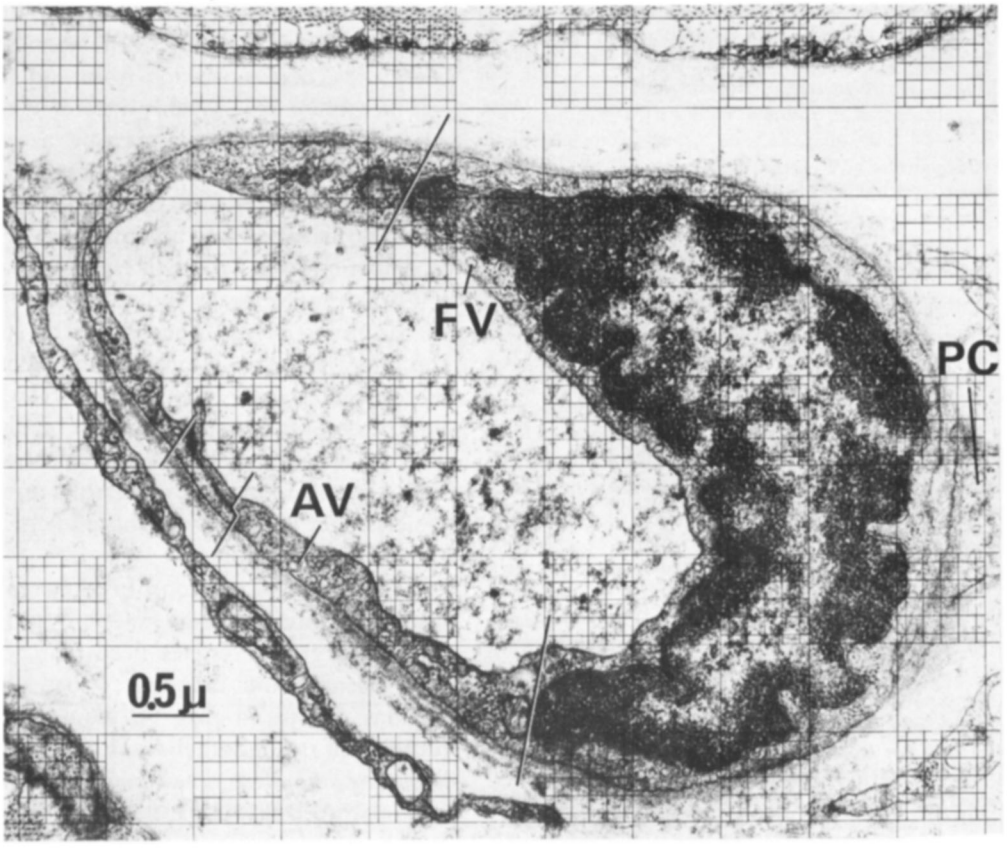

FIG. 1. Electron micrograph of a capillary from striated muscle of the rat showing attached vesicles (AV) and free vesicles (FV) in the vascular endothelial cell. The endothelium is divided into nuclear, junctional, and peripheral zones, as defined in the text. PC indicates a pericyte profile. Superimposed on the micrograph is the double-square grid employed for the morphometric analysis. Its coarse lattice has an actual distance between the lines of 25 mm.

RESULTS

The number of MPV was significantly larger in the diabetic rats injected with insulin (D_i) than in the saline-injected diabetic rats (D). The numeric density of all vesicles, i.e., free + attached, per cross-sectional area of total endothelial cytoplasm was 44.2 ± 8.5 and 34.0 ± 4.5/μ^2 (mean ± S.D.) in the two groups, respectively, 2p = 0.045 (figure 2). There were no differences between insulin-injected rats and non-diabetic controls (C), the mean value in the latter group being 48.0 ± 7.3/μ^2, 2p = 0.51. The two reference groups D and C also showed a statistically significant difference, 2p = 0.0097. Further analysis showed that there was a marked difference in the distribution between free and attached vesicles in the groups. Compared to reference group C the number of free vesicles was reduced by 40 per cent in saline-injected diabetic rats, whereas that of the less frequently occurring attached vesicles was increased by 50 per cent (table 1). The insulin-injected diabetic animals, on the other hand, showed almost normal absolute values. The ratio free/attached vesicular profiles in the peripheral zone of the endothelial cells was significantly higher in D_i than in D (7.6 ± 1.7 and 4.3 ± 0.9, respectively, 2p = 0.022) and also higher in C (11.4 ± 2.2, 2p = 0.024) than in D_i (figure 3).

As it is well known from other studies[9,13] the number of vesicles attached to the abluminal cell membrane exceeds that of the luminal membrane. In the present series it was found that the ratio between vesicles attached luminally and abluminally was higher in the saline-injected diabetic rats than in non-diabetic control rats (table 1).

Various parameters for capillary characteristics, i.e., the thicknesses of endothelial cytoplasm, capillary diameter, and fractional volume of the three individual zones (peripheral, junctional, nuclear) were identical in the three groups (table 1). The size of the vesicular profiles also failed to show any differences between the groups.

DISCUSSION

It is generally agreed that the vesicles move about

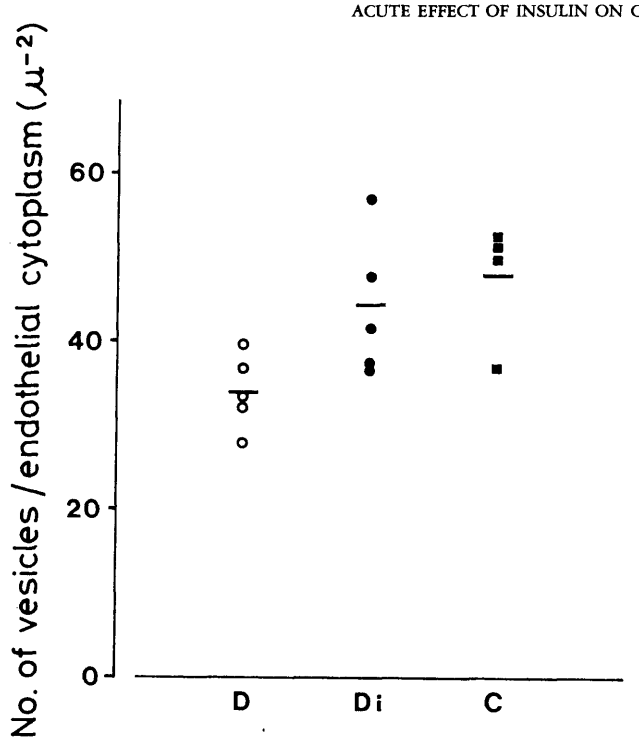

FIG. 2. Numeric density of micropinocytotic vesicular profiles (free + attached) per cross-sectional area of total endothelial cytoplasm exclusive of the nucleus. Values shown are for untreated diabetic rats (D), diabetic rats given insulin about 60 minutes earlier (Di), and control rats (C). Bars indicate mean values.

freely and randomly in the cytoplasm driven by purely physical (Brownian) forces independent of cellular energy.[12] Their separation from and fusion with the cell membrane are probably a result of the same forces, whereas cellular energy may be necessary for their formation from the cell membrane. The small total number of vesicles and the markedly abnormal distribution between free and attached vesicles observed in diabetic rats are most likely due to a change in the physical properties of the cell membrane, a change that is almost restored one hour after insulin administration. The information available about the kinetics of vesicular transport is limited. However, considering the observation that transport of albumin from the vascular space is increased after insulin,[5] it is tempting to speculate that the increase in numeric density of vesicles after insulin is the morphologic counterpart of this effect of insulin.

Although the relationship between the action of insulin on cardiovascular function[5] and on endothelial micropinocytotic vesicles needs further clarification, the present results do suggest that adequate concentrations of insulin may be required for the normal function of the endothelial cells. The finding reported here may, therefore, be of relevance to diabetic microangiopathy, considering the close structural relationship between endothelial cells and capillary basement

TABLE 1

Morphometric data on rat-muscle capillaries and endothelial vesicles in the peripheral zone

	Diabetic + saline (n = 5)		Diabetic + insulin (n = 5)	Control + saline (n = 4)
Arithmetic mean endothelial thickness[16] (surface density^{-1}, μ)	0.19 ± 0.02*		0.19 ± 0.02	0.20 ± 0.02
Mean capillary (cylinder) diameter[17] (average mean net curvature^{-1}, μ)	3.6 ± 0.7		3.6 ± 0.7	3.0 ± 0.2
Fractional volume of peripheral zone (per cent of cytoplasm)	73 ± 11		74 ± 8	80 ± 4
Mean diameter of vesicular profiles (2 (mean profile area/π)$^{1/2}$, nm.)	48 ± 7		48 ± 4	49 ± 4
Ratio between numbers of vesicular profiles attached to luminal and abluminal membrane	0.58 ± 0.04		0.54 ± 0.13	0.43 ± 0.08†
Numeric density of free vesicular profiles (no./μ)‡	1.0 ± 0.2	2 p=0.015**	1.4 ± 0.2	1.6 ± 0.2§
Numeric density of attached vesicular profiles (no./μ)‡	0.23 ± 0.02	2 p=0.022**	0.19 ± 0.03	0.15 ± 0.04//

*Mean ± S.D. (between animals).
†Different from diabetics + saline, 2p = 0.0077, but not from diabetics + insulin, 2p = 0.21.
‡Numbers of profiles per total length of cell membrane (abluminal + luminal).
§Different from diabetics + saline, 2p = 0.0050, but not from diabetics + insulin, 2p = 0.18.
//Different from diabetics + saline, 2p = 0.0048, but not from diabetics + insulin, 2p = 0.13. All other differences are nonsignificant.
**The 2p-value of the difference between diabetic + saline and diabetic + insulin.

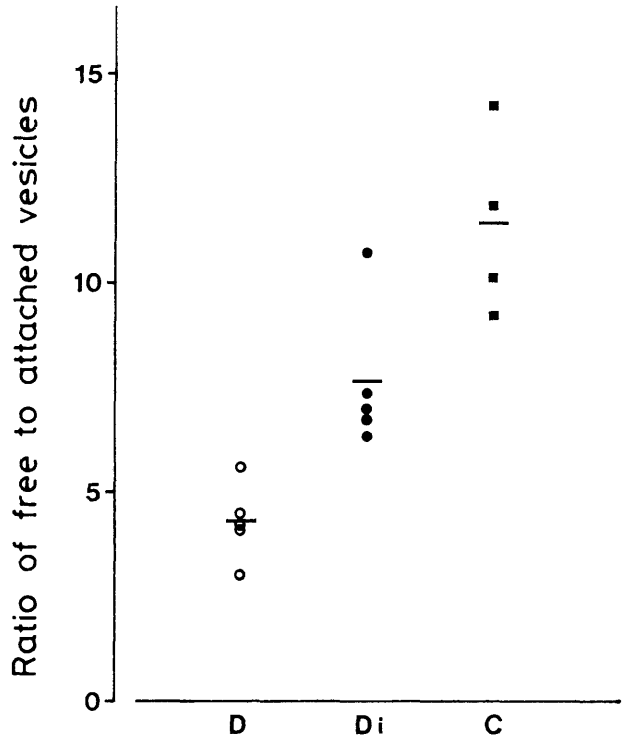

FIG. 3. Ratio between the number of free and attached micropinocytotic vesicular profiles in the peripheral zone of endothelial cells. Signatures as in figure 2.

membrane. Thickening of the capillary basement membrane is the most important morphologic feature of diabetic microangiopathy.[14,15]

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