to reactive granules (mono and pluri) were indicators that the antibodies were of adequate specificity.

1 Kalina, M., Elmalek, M., and Hammel, I., Histochemistry *89* (1988) 193.
2 Kovacs, K., and Horvath, E., Tumours of the pituitary gland. Atlas of tumor pathology, 2nd ser., Fascicle 21. Armed Forces Institute of Pathology, Washington, D.C. 1986.
3 Kurosumi, K., Tanaka, S., and Tosaka, H., Arch. Histol. Jap. *50* (1987) 455.
4 Pelletier, G., Robert, F., and Hardy, J., J. clin. Endocr. Metab. *46* (1978) 133.
5 Lloyd, R., Cano, M., Chandler, W., Barkan, A., Vorvath, E., and Kovacs, K., Am. J. Path. *134* (1988) 605.
6 Robert, F., Pelletier, G., Serri, O., and Hardy, J., Hum. Path. *19* (1988) 99–104.
7 Beckers, A., Courtoy, R., Stevenaert, A., Boniver, J., Closset, J., Frankenne, F., Reznik, M., and Hennen, G., Acta endocr. (Copenh.) *118* (1988) 503.
8 Whitnall, M. H., Gainer, H., Cox, B. M., and Molineaux, Cj., Science *222* (1983) 1137.
9 Childs, G. V., Yang, H.-Y., Tobin, R. B., Wilber, J. F., and Kubek, M., J. Histochem. Cytochem. *29* (1981) 357.
10 Lehy, T., Peranzi, G., and Cristina, M. L., Histochemistry *71* (1981) 67.
11 Moriarty, G., and Garner, L., Nature *265* (1977) 366.
12 Childs, G. V., Unabia, G., Tibolt, R., and Lloyd, J. M., Endocrinology *121* (1987) 1801.
13 Nikitovitch-Winer, M. B., Atkin, J., and Maley, B. E., Endocrinology *121* (1987) 625.
14 Tanaka, S., and Kurosumi, K., Cell Tissue Res. *243* (1986) 229.
15 Frawley, L. S., and Neill, J. D., in: Proceedings of the 65th Annual Meeting of the Endocrine Society, p. 310 (abstract) 1983.
16 Fumagalli, G., and Zanini, A., J. Cell Biol. *100* (1985) 2019.
17 Ishibashi, T., and Shino, M., Anat. Rec. *223* (1989) 185.
18 Scheithauer, B. W., Horvath, E., Kovacs, K., Laws, E. R., Randall, R. V., and Ryan, N., Semin. Diagn. Path. *3* (1986) 69.
19 Heitz, P. U., Landolt, A. M., Zenklusen, H. R., Kasper, M., Reubi, J. C., Oberholzer, M., and Roth, J., J. Histochem. Cytochem. *35* (1987) 1005.
20 Zurschmiede, C., and Landolt, A. M., Virchows Arch. (B) *53* (1987) 308.
21 Felix, A., Horvath, E., Kovacs, K., Smyth, H. S., Killinger, D. W., and Vale, J., Acta neuropath. (Berl). *71* (1986) 76.
22 Holm, R., and Nesland, J. M., Ultrastruct. Path. *12* (1988) 279.
23 Newman, G. R., Jasani, B., and William, E. D., J. Histochem. Cytochem. *37* (1989) 1183.
24 Asa, S. L., Kovacs, K., Horvath, E., Losinski, N. E., Laszlo, F. A., Domokos, I., and Halliday, W. C., Neuroendocrinology *48* (1988) 423.
25 Suzukawa, K., Kawaoi, A., Sato, K., and Ishii, S., Acta path. jap. *38* (1988) 153.
26 Asa, S. L., Penz, G., Kovacs, K., and Ezrin, C., Arch. Path. Lab. Med. *106* (1982) 360.
27 Losinski, N. E., Horvath, E., and Kovacs, K., Am. J. Anat. *185* (1989) 236.
28 Malagon, M. M., Garcia-Navarro, S., Garcia-Herdugo, G., and Garcia-Navarro, F., Histochemistry *89* (1988) 461.
29 Tasca, C., Morph. Embriol. *34* (1988) 189.
30 Childs, G. V., Ellison, D. G., Ramaley, J. A., and Unabia, G., Endocrinology *110* (1982) 1676.
31 Childs, G. V., Ellison, D. G., Lorenzen, J. R., Collins, T. J., and Swartz, N. B., Endocrinology *113* (1983 a) 166.
32 Childs, G. V., Hyde, C., Naor, Z., and Catt, K., Endocrinology *113* (1983 b) 2120.
33 Childs, G. V., Westland, K. N., and Unabia, G., Endocrinology *125* (1989) 554.
34 Newman, G. R., Jasani, B., and Williams, E. D., J. Histochem. Cytochem. *18* (1986) 67.
35 Slater, M. D., Stain Technology (1990) in press.
36 Childs, G. V., in: Endocrinology, p. 499. Eds F. LaBrie and A. Proulx. Scientific, Amsterdam/New York 1985.

0014-4754/91/030267-04$1.50 + 0.20/0

Effect of hyperkalemia on insulin secretion

R. Martinez, B. Rietberg, J. Skyler, J. R. Oster and G. O. Perez*

Medical and Research Services, Veterans Administration Medical Center and the Department of Medicine, University of Miami School of Medicine, Miami (Florida 33125, USA)
Received 22 May 1990; accepted 20 August 1990

Summary. The effect of hyperkalemia on insulin secretion remains undefined. We evaluated portal and peripheral insulin levels in anesthetized dogs after infusions of KCl. The mean maximal increase in peripheral plasma potassium at infusion rates of 0.2 mEq/kg/h was 0.68 ± 0.20 mEq/l. There were no significant increases in either portal or peripheral insulin levels. In contrast, in six dogs whose plasma potassium concentration increased in each case by more than 2.0 mEq/l (infusion rate of 0.5 mEq/kg/h), portal insulin levels increased fivefold ($p < 0.05$). We conclude that only marked increases in plasma potassium concentration stimulate pancreatic insulin secretion.
Key words. Insulin; potassium; hyperkalemia; portal vein; glucose.

It is well established that insulin promotes cellular potassium uptake and lowers blood potassium concentration [1–5] and that a basal blood level is necessary to allow normal potassium tolerance [6]. The effect of hyperkalemia on insulin secretion remains undefined, however. The results of several studies [4, 5, 7, 8] suggest that stimulation of insulin production is only observed in the presence of marked increases in serum potassium concentration (e.g., greater than 1–2 mEq/l). Unfortunately, in these studies, sampling from the portal circulation was not performed. Thus, it is possible that smaller increments in plasma potassium enhanced insulin secretion, which was masked because of the capacity of the liver to extract insulin. In the present study, we evaluated the pancreatic insulin response to graded degrees of hyperkalemia in anesthetized dogs with intact kidneys. The results validate the

previously held impression that only marked increases of serum potassium concentration stimulate the pancreatic secretion of insulin.

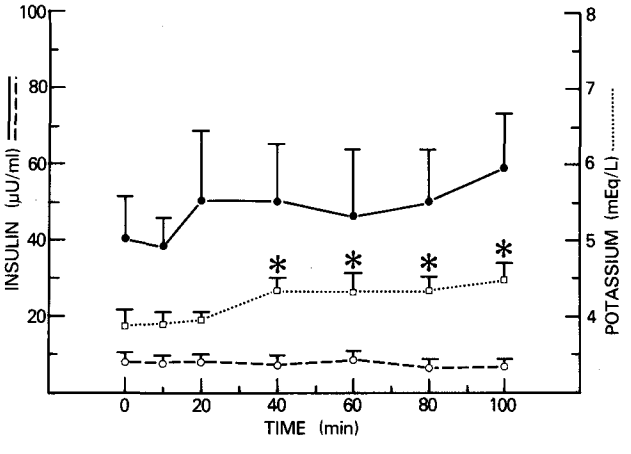
Materials and methods

Experiment protocol. The studies were performed in sixteen male or female mongrel dogs weighing 18–22 kg. The day of the experiment, after an overnight fast, general anesthesia was induced with pentobarbital (30 mg/kg b.wt i.v.) and constant ventilation achieved with a Harvard respirator. Additional small amounts of pentobarbital were administered when necessary. After a midline incision, a catheter was placed in the portal vein. Additional catheters were placed in the saphenous vein for potassium infusion and in the contralateral femoral artery and femoral vein for blood pressure monitoring and blood sampling, respectively. The catheters were flushed with 0.5 ml heparinized saline solution (1 μU/ml) to assure their patency.

After 45 min equilibration, three baseline blood samples were obtained from the portal and femoral veins for potassium, glucose, and insulin determinations. In seven dogs, KCl was then infused at a rate of 1 ml/min in amounts calculated to deliver 0.2 mEq of potassium/kg b.wt/h for 100 min. The KCl was diluted in 0.45% NaCl, and the potassium concentration in the infusate ranged from 70 to 80 mEq/l. Blood samples were obtained every 10 min for the first two collection periods and subsequently at 20-min intervals. In six additional animals, a higher rate of infusion of 0.5 mEq/kg b.wt/h was used in order to increase peripheral vein potassium levels by at least 1.0 mEq/l. Finally in three control dogs, the experiment was repeated by infusing 0.45% NaCl as above, without KCl, in order to assess the effect of abdominal manipulation.

Laboratory methods. Blood samples were analyzed for potassium by flame photometry and for glucose utilizing an autoanalyzer (Technicon Instruments, Tarrytown, New York). Insulin was measured by radioimmunoassay.

Statistical analysis. The data shown in the figure and table 1 were analyzed by analysis of variance followed by the paired t-test to test for changes in serum concentration from baseline.



Changes in portal vein insulin levels (closed circles) and peripheral vein insulin levels (open circles) in seven dogs infused with KCl (0.2 mEq/kg/h). Peripheral plasma potassium levels are depicted by open squares. The vertical lines represent one SE of the mean. The asterisks denote changes that are significantly different from baseline ($p < 0.05$).

Results

No statistically significant changes from baseline values were observed during KCl infusion in mean femoral blood pressure recordings obtained with a pressure transducer.

As shown in the figure, there was no significant change in portal vein insulin levels during 100 min of infusion in the seven dogs receiving KCl at 0.2 mEq/kg/h. The mean maximal increase in peripheral plasma potassium levels in these animals was 0.68 ± 0.2 (SE) mEq/l. Baseline peripheral insulin levels were low (8.1 ± 1.5 μU/ml) and did not change throughout the experiments. Portal potassium levels tended to exceed those in the peripheral vein during the baseline period and during the initial 20 min of the experiment. Subsequently, the levels in the peripheral vein were slightly higher than those in portal blood. Peripheral plasma glucose concentration was 104 ± 4 mg/dl at baseline, and the levels did not change during the experiment.

Table 1 shows the peripheral plasma potassium levels and the levels of insulin in the portal vein in the six animals infused with KCl at 0.5 mEq/kg/h. In each dog, the increment in peripheral plasma potassium was greater than 2.0 mEq/l. Portal vein insulin increased markedly in each animal but peripheral insulin levels were undetectable in two animals and increased only in one dog.

The levels of potassium and insulin in both peripheral vein and portal vein in control animals (not infused with potassium) are shown in table 2. In these animals, potassium and insulin levels remained unchanged or decreased during the experiment.

Discussion

Insulin promotes potassium transfer from ECF to ICF independent of glucose uptake by increasing the activity

Table 1. The effect of high rate KCl infusion (0.5 mEq/l/kg/h) on peripheral and portal insulin levels in six dogs

Time (min)	K^+ (mEq/l) Peripheral	Portal	Insulin (μU/ml) Peripheral	Portal
0	4.23 ± 0.14	4.30 ± 0.07	6.3 ± 1.2	31.9 ± 12
10	4.59 ± 0.02*	4.52 ± 0.7†	5.3 ± 0.81	27.2 ± 6
20	4.78 ± 0.07*	4.79 ± 0.09†	5.9 ± 0.68	50.6 ± 18
40	5.27 ± 0.01†	5.04 ± 0.08†	6.4 ± 0.34	58.0 ± 11
60	5.85 ± 0.20†	5.82 ± 0.30†	9.1 ± 1.5	103 ± 30
80	6.36 ± 0.38†	6.43 ± 0.45†	18.5 ± 5.9	105 ± 24
100	7.05 ± 0.49†	7.11 ± 0.56†	16.2 ± 5.2	150 ± 31*

Data are $\bar{X}$ ± SE; *$p < 0.05$; †$p < 0.005$ from baseline.

Table 2. Potassium and insulin levels in three control dogs that did not receive potassium infusions

Time (min)	K^+ (mEq/l) Peripheral				Portal				Insulin (μU/ml) Peripheral				Portal			
	Dog 1	Dog 2	Dog 3	$\bar{X} \pm SE$	Dog 1	Dog 2	Dog 3	$\bar{X} \pm SE$	Dog 1	Dog 2	Dog* 3	$\bar{X} \pm SE$	Dog 1	Dog 2	Dog 3	$\bar{X} \pm SE$
0	4.77	3.63	3.66	4.02 ± 0.37	4.72	3.54	3.64	3.97 ± 0.38	4.6	11.0	< 2	5.6 ± 2.8	30.3	106	14.4	50.2 ± 28.2
10	4.90	3.60	3.69	4.06 ± 0.42	4.81	3.52	3.67	4.00 ± 0.40	4.0	7.0	< 2	4.2 ± 1.6	15.1	121	11.2	49.1 ± 35.0
20	4.90	3.64	3.49	4.01 ± 0.45	4.91	3.61	3.63	4.05 ± 0.43	4.6	8.0	< 2	4.7 ± 1.8	21.7	107	22.8	50.5 ± 28.2
40	4.95	3.43	3.39	3.92 ± 0.51	4.90	3.40	3.40	3.90 ± 0.50	7.0	5.2	< 2	4.6 ± 1.6	20.9	27.2	9.6	19.2 ± 5.1
60	4.82	3.64	3.26	3.90 ± 0.47	4.92	3.55	3.27	3.91 ± 0.51	2.4	7.0	< 2	3.6 ± 1.7	14.9	43.2	18.4	25.5 ± 8.9
80	4.91	3.56	3.24	3.90 ± 0.51	4.67	3.39	3.27	3.77 ± 0.45	1.8	5.4	< 2	2.9 ± 1.2	12.6	36.1	30.4	26.3 ± 7.1
100	4.70	3.40	3.20	3.83 ± 0.43	4.64	3.20	3.26	3.70 ± 0.47	2.5	5.5	< 2	3.2 ± 1.2	15.0	45.8	13.9	24.9 ± 10.4

* $\bar{X}$ calculated assuming a value of 1.5 μU/ml for peripheral insulin values of dog N° 3.

of $(Na^+ + K^+)$-ATPase[9]. Furthermore, there is evidence that hyperkalemia may stimulate insulin release from the pancreas[1–8]. These studies indicated that this response occurs only after substantial increments in plasma potassium concentration (greater than 1 to 2 mEq/l), and that it is not observed with lesser changes in plasma potassium levels.

Because of the substantial hepatic extraction of insulin, the sampling from peripheral vein utilized in the previous studies might have missed small increases of insulin secretion. In the present study, we simultaneously measured both peripheral and portal insulin levels following KCl infusions designed to produce relatively small increases of plasma potassium concentration (from 0.3 to 0.8 mEq/l). There was a tendency for portal insulin levels to increase but the results did not achieve statistical significance. No significant changes in peripheral insulin levels were observed. On the other hand, portal insulin levels increased markedly in the dogs infused with a greater amount of potassium so that plasma potassium levels increased by more than 2.0 mEq/l. Because of substantial hepatic extraction peripheral insulin levels increased in only one of these animals.

In one previous study[10], sampling for insulin from the posterior superior duodenal veins was performed in four dogs infused with KCl. Three dogs had a bilateral nephrectomy, however. In these animals, increases in plasma potassium concentration of 0.6–1.0 mEq/l resulted in increases in both central and peripheral insulin levels. In the only dog with intact kidneys, insulin levels increased from 82 to 202 μU/ml five min after the beginning of the experiment while plasma potassium increased only from 4.8 to 5.1 mEq/l.

The present findings suggest that relatively small changes in plasma potassium concentration (less than 1 mEq/l) do not result in significant changes in plasma insulin levels, whereas marked increases (greater than 2 mEq/l) stimulate insulin release by the pancreas.

Acknowledgments. The authors wish to thank Melanie Ashby for her technical assistance, and Gilda Manicourt and Judy Hunter for their secretarial expertise.

* Please address all correspondence to G. P., Dialysis Unit (III C), Veterans Administration Medical Center, 1201 N.W. 16th Street, Miami, Florida 33125.

1 Silva, P., Brown, R. S., and Epstein, F. H., Kidney Int. *11* (1977) 466.
2 DeFronzo, R. A., and Bia, M., in: The Kidney; Physiology and Pathophysiology, p. 1179. Eds D. W. Seldin and G. Giebisch. Raven Press, New York 1985.
3 Hiatt, N., Yamakawa, T., and Davidson, M. B., Metabolism *23* (1974) 43.
4 Hiatt, N., Chapman, L. C., Davidson, M. B., Skeinkope, J. A., and Miller, A., Am. J. Physiol. *231* (1976) 1660.
5 Pettit, G., Vick, R. L., and Swander, A. M., Am. J. Physiol. *228* (1975) 197.
6 DeFronzo, R. A., Sherwin, R. S., Dilligham, M., Hendler, R., Taborlane, W. V., and Felig, P., J. clin. Invest. *61* (1978) 472.
7 Santeusano, F., Faloona, G., Knochel, J. P., and Ungar, R. H., J. Lab. clin. Med. *81* (1973) 809.
8 Dluhy, R. G., Axelrod, L., and Williams, G. H., J. appl. Physiol. *33* (1972) 22.
9 Moore, R. D., Biochim. biophys. Acta *737* (1983) 1.
10 Hiatt, N., Davidson, M. B., and Bonorris, G., Horm. Metab. Res. *4* (1972) 64.

0014-4754/91/030270-03$1.50 + 0.20/0