1 B. BLOMBÄCK, M. BLOMBÄCK, P. EDMAN AND B. HESSEL, *Nature*, 193 (1962) 883.
2 B. BLOMBÄCK AND M. BLOMBÄCK, *Arkiv Kemi*, 10 (1956) 415.
3 B. BLOMBÄCK AND A. VESTERMARK, *Arkiv Kemi*, 12 (1958) 173.
4 B. BLOMBÄCK, M. BLOMBÄCK, P. EDMAN AND B. HESSEL, in preparation.
5 B. BLOMBÄCK, *Arkiv Kemi*, 12 (1958) 99.
6 W. H. SEEGERS, E. C. LOOMIS AND J. M. VANDENBELT, *Arch. Biochem.*, 6 (1945) 85.
7 B. BLOMBÄCK AND I. YAMASHINA, *Arkiv Kemi*, 12 (1958) 299.
8 L. ENGSTRÖM, *Biochim. Biophys. Acta*, 52 (1961) 36.
9 W. D. BROWN, *Australian J. Exptl. Biol. Med. Sci.*, 32 (1954) 677.
10 G. E. PERLMANN, *Advan. Protein Chem.*, 10 (1955) 1.
11 G. E. PERLMANN, *J. Gen. Physiol.*, 41 (1958) 441.
12 J. B. MARTIN AND D. M. DOTY, *Anal. Chem.*, 21 (1949) 965.
13 O. LINDBERG AND L. ERNSTER, *Methods Biochem. Anal.*, 3 (1956) 16.

Received November 19th, 1962

Biochim. Biophys. Acta, 74 (1963) 148–151

SC 2244

Stimulation of glucose oxidation without glycogen deposition by oxytocin and vasopressin

It has recently been shown that the posterior pituitary hormones oxytocin and vasopressin share some of the metabolic properties of insulin[1,2]. All three substances stimulate glucose uptake from the incubation medium, oxidation of labeled glucose to $^{14}CO_2$, and incorporation of the ^{14}C of labeled glucose into lipids. Using a number of synthetic oxytocin analogues MIRSKY AND PERISUTTI showed that the insulin-like action on fat appeared to parallel the oxytocic activity and that not all these substances possessed such activity. Because the stimulation of glycogen deposition may represent a more characteristic action of insulin, we have evaluated oxytocin and vasopressin for this activity while simultaneously measuring glucose oxidation. The results indicate that the action of the posterior pituitary peptides is in some way different from that of insulin, despite their other similarities.

Epididymal fat pads were obtained from *ad libitum* fed Sprague–Dawley rats weighing approx. 200 g and were incubated as previously described[1,2] with the following modifications. The arrangement of the three segments of each fat pad was such that the control and each concentration of the test substances used were incubated with one proximal segment, one middle segment, and one distal segment. This was necessary to avoid bias due to the greater activity of the proximal segments. All incubations were conducted in 25- or 30-ml flasks (uniform in any experiment) fitted with a removable center well and were carried out for 1 h at 37° in a Dubnoff shaking incubator. The labeled substrate D-[1-^{14}C]glucose (New England Nuclear Corp.) was added at an activity of 0.25 μC/ml and a concentration of 1 mg/ml in a total volume of 2 ml of Krebs–bicarbonate buffer. At the end of the incubation period 2.0 ml of 4% trichloroacetic acid were injected through the rubber cap into the incubation medium and 1 ml of "Hyamine" base (Packard Instrument Company) was injected into the center well. The flasks were then incubated for another hour at room temperature, after which the center well was removed and the "Hyamine" transferred into a counting vial containing scintillator (2,5-diphenyloxazole, 0.5%, 1,4-bis-2-(4-methyl-5-phenyl-oxazolyl)-benzene, 0.03%) in toluene and counted in a

TABLE I

EFFECT OF OXYTOCIN AND VASOPRESSIN ON GLUCOSE METABOLISM

Expt.	Substance	Concentration (mg or micro-units/ml)	Approximate concn. (M)	$^{14}CO_2$ (counts/min/g)*	Glycogen deposition	
					(μg/g)*	(counts/min/g)*
1	Oxytocin	control	0	104 984 ± 2 100	436 ± 170	1440 ± 158
		$5 \cdot 10^{-4}$ mg	$5.0 \cdot 10^{-7}$	174 606 ± 6700	361 ± 75	1361 ± 180
		$5 \cdot 10^{-3}$ mg	$5.0 \cdot 10^{-6}$	249 859 ± 21 000	303 ± 150	1730 ± 180
		$5 \cdot 10^{-2}$ mg	$5.0 \cdot 10^{-5}$	224 189 ± 12 000	273 ± 70	1787 ± 170
		$5 \cdot 10^{-1}$ mg	$5.0 \cdot 10^{-4}$	247 424 ± 46 000	254 ± 158	1323 ± 120
	Insulin	250 micro-units	$2 \cdot 10^{-9}$	393 660 ± 31 200	577 ± 180	6754 ± 1250
2	Oxytocin	control	0	176 124 ± 11 400	246 ± 35	988 ± 47
		$5 \cdot 10^{-4}$ mg	$5.0 \cdot 10^{-7}$	224 240 ± 18 300	198 ± 33	969 ± 28
		$5 \cdot 10^{-3}$ mg	$5.0 \cdot 10^{-6}$	273 621 ± 36 000	146 ± 10	813 ± 50
		$5 \cdot 10^{-2}$ mg	$5.0 \cdot 10^{-5}$	268 371 ± 23 500	215 ± 10	947 ± 134
		$5 \cdot 10^{-1}$ mg	$5.0 \cdot 10^{-4}$	265 534 ± 28 200	161 ± 17	632 ± 60
	Insulin	250 micro-units	$2 \cdot 10^{-9}$	527 173 ± 51 000	351 ± 43	4794 ± 400
3	Oxytocin	control	0	154 206 ± 30 000		3149 ± 370
		$5 \cdot 10^{-5}$ mg	$5.0 \cdot 10^{-8}$	160 478 ± 22 300		2568 ± 360
		$5 \cdot 10^{-4}$ mg	$5.0 \cdot 10^{-7}$	228 734 ± 30 000		2569 ± 235
		$5 \cdot 10^{-3}$ mg	$5.0 \cdot 10^{-6}$	263 236 ± 49 000		2578 ± 201
		$5 \cdot 10^{-2}$ mg	$5.0 \cdot 10^{-5}$	340 468 ± 50 000		3572 ± 520
	Insulin	250 micro-units	$2 \cdot 10^{-9}$	500 083 ± 104 000		10 658 ± 2000
4	Lysine vasopressin (Sandoz)	control	0	155 011 ± 8500	187 ± 29	1833 ± 106
		$5 \cdot 10^{-5}$ mg	$5.0 \cdot 10^{-8}$	236 404 ± 30 000	123 ± 5	2060 ± 60
		$5 \cdot 10^{-3}$ mg	$5.0 \cdot 10^{-6}$	337 024 ± 50 000	147 ± 25	2084 ± 150
		$5 \cdot 10^{-2}$ mg	$5.0 \cdot 10^{-5}$	276 247 ± 47 000	81 ± 10	1610 ± 39
		$5 \cdot 10^{-1}$ mg	$5.0 \cdot 10^{-4}$	55 717 ± 12 500	111 ± 12	1221 ± 71
	Insulin	250 micro-units	$2 \cdot 10^{-9}$	319 594 ± 46 000	381 ± 90	26 775 ± 3500
5	Lysine vasopressin (NIH)	control	0	156 745 ± 35 000		2286 ± 150
		$5 \cdot 10^{-5}$ mg	$4.4 \cdot 10^{-8}$	129 032 ± 20 000		2206 ± 115
		$5 \cdot 10^{-3}$ mg	$4.4 \cdot 10^{-6}$	168 166 ± 16 000		2523 ± 240
		$5 \cdot 10^{-2}$ mg	$4.4 \cdot 10^{-5}$	202 174 ± 40 000		2503 ± 240
		$5 \cdot 10^{-1}$ mg	$4.4 \cdot 10^{-4}$	165 632 ± 8500		2003 ± 150
	Insulin	250 micro-units	$2 \cdot 10^{-9}$	265 493 ± 10 500		12 783 ± 1170

* Mean of 3 values ± standard error of the mean.

Tri-Carb liquid scintillation counter. The tissue was homogenized in the medium and the homogenate heated in a boiling-water bath for 5 min, cooled to room temperature, and the trichloroacetic acid concentration adjusted to 5 %. This mixture was centrifuged and the glycogen precipitated at 4° overnight from the supernatant solution with 5 vol. of absolute ethanol and 1 ml of 10 % Na_2SO_4. The resulting precipitate was washed 3 times with absolute ethanol, dissolved in 2 ml of 50 % KOH, and boiled for 15 min. This KOH solution was then divided into two aliquots, the glycogen precipitated from each with ethanol, and one used for counting as above and the other for the chemical determination of glycogen[3].

The results of 5 experiments are shown in Table I. It is seen that oxytocin and vasopressin are capable of stimulating oxidation of [1-^{14}C]glucose to $^{14}CO_2$ without enhancing the deposition of glycogen under conditions in which insulin markedly stimulates glycogen deposition as measured both chemically and by incorporation of radioactivity. In 11 other experiments, which were similar except that the glycogen

and $^{14}CO_2$ determinations were not made simultaneously, oxytocin appeared to stimulate glucose oxidation more consistently than did vasopressin, but in no case did either increase glycogen deposition over control values. In agreement with the report of MIRSKY AND PERISUTTI[2], we found that phenylalanyl-2-lysyl-8-vasopressin failed to stimulate glucose oxidation.

An interesting finding was the apparently graded decrease in glycogen deposited with increasing concentrations of oxytocin or vasopressin. However, this was not a consistent finding, since it did not occur in all the other experiments, and only further work can tell whether it is of biological significance. It is possible that this represents glycogenolysis stimulated by the peptides. However, there was never any rise in the glycogen content under the influence of the pituitary peptides as compared with control values. We have no data to evaluate the possibility that glucose is in fact incorporated into glycogen at an accelerated rate under the influence of the pituitary peptides but that this effect is obscured by a concomitant glycogenolysis. Although incubation for longer periods or with different buffers[4] might permit demonstration of some glycogen-depositing activity, this seems unlikely in view of the present data; and, in any case the pituitary hormones show a marked dissociation of potencies with respect to glucose oxidation and glycogen deposition.

Since the posterior pituitary peptides can simulate several of the actions of insulin on adipose tissue[1,2], as mentioned above, and since variations of the eigth, ninth, and tenth amino acids of the HA-chain of insulin (within the A6 to A11 disulfide ring) appear to leave the biological activity of the molecule intact[5], it was thought that vasopressin and oxytocin might reproduce the action of insulin rather closely. However, the present results show that despite superficial similarities, the action of insulin is in some way different from that of the posterior pituitary hormones. Since both oxytocin and insulin stimulate glucose oxidation and stimulate the oxidation of the first carbon to a greater extent than the sixth[1,2], but since only insulin stimulates glycogen deposition, it would seem that insulin is doing something more "directive" than simply allowing more glucose to enter the intracellular metabolic pathways.

Mr. D. HAMNER and Miss P. HILL provided competent technical assistance. All the pituitary hormone preparations were synthetic and were supplied by Dr. R. BIRCHER, of the Sandoz Company, except a portion of the lysine vasopressin which was supplied by the Endocrinology Study Section of the National Institutes of Health (260 pressor units per mg). The crystalline, glucagon-free insulin was supplied by Dr. W. R. KIRTLEY of the Eli Lilly Company.

Radioisotope Service and Medical Service,
Birmingham Veterans Administration Hospital,
and Department of Medicine,
University of Alabama Medical Center,
Birmingham, Alabama (U.S.A.)

JAMES A. PITTMAN, Jr.
B. R. BOSHELL

[1] J. A. PITTMAN, B. R. BOSHELL, B. H. WILLIAMS, D. HAMNER AND P. HILL, *Biochem. Biophys. Res. Commun.*, 6 (1961) 29.
[2] I. A. MIRSKY AND G. PERISUTTI, *Endocrinol.*, 71 (1962) 158.
[3] R. W. HANSON, H. S. SCHWARTZ AND S. B. BARKER, *Am. J. Physiol.*, 198 (1960) 800.
[4] A. B. HASTINGS, C. T. TENG, F. B. NESBETT AND F. M. SINEX, *J. Biol. Chem.*, 194 (1952) 69.
[5] J. I. HARRIS, F. SANGER AND M. A. NAUGHTON, *Arch. Biochem. Biophys.*, 65 (1956) 427.

Received January 2nd, 1963