INSULIN-LIKE ACTIVITY OF VASOPRESSIN AND OXYTOCIN

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Received August 21, 1961

The presence of a hexapeptide disulfide ring in the A chain of insulin and in vasopressin (antidiuretic hormone, ADH) and oxytocin suggests that these substances may share some metabolic properties. The following experiments demonstrate that this is the case.

METHODS: Glucose oxidation was measured by production of $C^{14}O_2$ from D-glucose-1- or -6-C^{14}. Rat epididymal fat pads were divided into 6 pieces and each portion incubated at 37°C in 2 ml of Krebs bicarbonate buffer containing 1.0 or 1.5 mg/ml of unlabelled glucose and 0.25 or 0.5 μc of radioactive glucose. At 45 minutes the incubation was stopped, 0.2 ml of 10 N H_2SO_4 was added, and the $C^{14}O_2$ was trapped in Hyamine base.

Glucose uptake from the medium was measured by determining (Froesch et al, 1956) the difference between initial and final concentrations in the medium in preparations similar to those just described but which contained no radioisotopic glucose.

Free fatty acid (FFA) "release" into the medium was measured by determination of the FFA (Dole, 1956) content of the medium with similar preparations to which had been added 2 grams/100 ml of bovine serum albumin. No attempt was made to estimate the contribution of release from or utilization by the tissue to the resulting net concentration in the medium, although the tissues were homogenized and the final tissue concentrations of FFA were also determined.

All results were corrected for the weight of the tissue used. Since 3 or 4 rats were used in each experiment, each result reported represents the mean of 3 or 4 determinations.

RESULTS AND DISCUSSION: Representative results of experiments on the effect of

oxytocin and ADH on glucose oxidation (17 experiments) are shown in Table 1. Both hormones stimulate glucose oxidation, but oxytocin appears to stimulate C-1 more than

Table 1

Stimulation of Glucose Oxidation by Oxytocin and Vasopressin

Test Substance Added to Medium	Concentration in Medium mg/ml	approx. molarity*	Position of Label	$C^{14}O_2$ Trapped cpm/gram tissue	Tissue
----------(control)----------			C-1	29324	Adipose tissue
Oxytocin	10^{-6}	9.9×10^{-10}	C-1	34567	" "
"	10^{-5}	9.9×10^{-9}	C-1	38390	" "
"	10^{-4}	9.9×10^{-8}	C-1	37272	" "
"	10^{-3}	9.9×10^{-7}	C-1	45764	" "
"	10^{-2}	9.9×10^{-6}	C-1	56666	" "
----------(control)----------			C-6	7596	" "
Oxytocin	10^{-6}	9.9×10^{-10}	C-6	5785	" "
"	10^{-5}	9.9×10^{-9}	C-6	7536	" "
"	10^{-4}	9.9×10^{-8}	C-6	10870	" "
"	10^{-3}	9.9×10^{-7}	C-6	10364	" "
"	10^{-2}	9.9×10^{-6}	C-6	10481	" "
----------(control)----------			C-1	20101	Adipose tissue
Vasopressin	10^{-6}	1.2×10^{-10}	C-1	15959	" "
"	10^{-4}	1.2×10^{-8}	C-1	18886	" "
"	10^{-2}	1.2×10^{-6}	C-1	24840	" "
"	10^{-1}	1.2×10^{-5}	C-1	31927	" "
"	1	1.2×10^{-4}	C-1	42030	" "
----------(control)----------			C-6	8820	" "
Vasopressin	10^{-6}	1.2×10^{-10}	C-6	9265	" "
"	10^{-4}	1.2×10^{-8}	C-6	9176	" "
"	10^{-2}	1.2×10^{-6}	C-6	12607	" "
"	10^{-1}	1.2×10^{-5}	C-6	15779	" "
"	1	1.2×10^{-4}	C-6	47729	" "
----------(control)----------			C-1	15878	Adipose tissue
ADH	2×10^{-2}	1.2×10^{-6}	C-1	22625	" "
Insulin	15.5 μu/ml	1.2×10^{-10}	C-1	72809	" "
NEM		10^{-4}	C-1	15773	" "
ADH + NEM	(Same conc. as above)		C-1	15214	" "
Insulin + NEM	(" " ")		C-1	41530	" "
----------(control)----------			C-1	24227	Beef kidney cortex
ADH	5×10^{-4}	6.2×10^{-8}	C-1	19699	" " "
"	5×10^{-3}	6.2×10^{-7}	C-1	20222	" " "
"	10^{-2}	6.2×10^{-6}	C-1	21814	" " "
----------(control)----------			C-1	25650	Beef kidney medulla
ADH	5×10^{-4}	6.2×10^{-8}	C-1	27084	" " "
"	5×10^{-3}	6.2×10^{-7}	C-1	30254	" " "
"	10^{-2}	6.2×10^{-6}	C-1	26650	" " "

*Approximate molar concentrations were calculated for ADH from comparison of the activity of the preparation used with the specific activity of synthetic arginine vasopressin (Acher, 1960). In calculating the molarity for the insulin it was assumed that the crystalline insulin used was pure.

C-6 oxidation. In this respect oxytocin stimulation more closely resembles that obtained with insulin while that from ADH is more like the effect of epinephrine (Cahill et al, 1960) or adrenocorticotropic hormone (ACTH), thyrotropin (TSH), and other substances (Freinkel, 1961). The data in Table 1 also show that ADH does not exert a similar action on the kidney and that the effect on fat can be blocked by N-ethylmaleimide (NEM).

The data in Table 2 show that ADH can stimulate glucose uptake from the medium by fat, although insulin appears to be considerably more potent in this action. Furthermore, as is seen in Table 3, ADH effects a net release of free fatty acids into the medium.

Table 2

Stimulation of uptakes of glucose from medium by vasopressin* and insulin. Incubation was of four hours duration.

Substance added to medium		Micromoles glucose/gram fat
(Tissue only)		5.5
ADH 2×10^{-1} mg/ml	1.2×10^{-5} M	11.0
Insulin 15.5 μu/ml	1.2×10^{-10} M	7.3
ADH, 2 mg/ml	2.5×10^{-4} M	14.0
Insulin 250 μu/ml	2×10^{-9} M	17.5

*Second batch ADH

Table 3

Stimulation of free fatty acid "release" into medium by vasopressin*. Incubation was for four hours

Substance added to medium		Total FFA at End of Incubation (microequivalents as palmitic acid)	
		In Medium (total)	In Tissue (per gram)
----------(tissue only)		1.16	10.1
ADH, 2×10^{-1} mg/ml	1.2×10^{-5} M	3.11	11.9
ADH, 2 mg/ml	1.2×10^{-4} M	6.68	10.9
Insulin 15.5 μu/ml	1.2×10^{-10} M	1.26	11.1
Insulin 115 μu/ml	1.0×10^{-9} M	3.89	15.0

*Second batch of ADH used

The fact that ADH can simulate three of the actions of insulin on fat and oxytocin at least one of these suggests that these substances might share a common mode of action.

The inhibition of the ADH effect on glucose oxidation by NEM (Table 1) further suggests that the formation of a sulfur-sulfur bond between the hormone and some tissue receptor might be involved similar to the mechanism recently postulated for its action on the kidney (Fong et al, Rasmussen et al, and Schwartz et al, 1961). However, the stimulation may be more non-specific, since strongly reductive conditions can also mimic insulin action (Lynn et al, 1961). Although the exact similarities and differences between the actions of the posterior pituitary hormones and insulin remain to be explored, the above data demonstrate that they do share some metabolic actions.

Acknowledgments

The vasopressin used was a preparation purified (80 pressor units/mg) from both beef and hog pituitaries from the Parke-Davis Company, kindly supplied by Mr. H.R. Layson. A second batch of ADH contained 40 pressor units/mg. The oxytocin was synthetic, prepared at the Sandoz Company and supplied by Dr. R. Bircher. It was assayed by the producer to contain 450 units/mg of the nonapeptide in one case and 398 in another. The crystalline insulin was generously supplied by Dr. W.R. Kirtley of the Lilly Research Laboratories, Indianapolis, Indiana. All other materials were obtained from commercial sources.

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