RAPID INCREASE OF INOSITOL 1,4,5-TRISPHOSPHATE CONTENT IN ISOLATED RAT ADIPOSE TISSUE BY VANADATE

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Sodium orthovanadate increased inositol 1,4,5-trisphosphate content in rat adipose tissue in a time-dependent manner up to 30 s; its maximal effect was two-fold higher than the basal level of IP_3, and it probably consequently causes elevation of the intracellular Ca^{2+}.

KEYWORDS inositol 1,4,5-trisphosphate; vanadate; Ca^{2+} release; adipose tissue

Sodium orthovanadate (vanadate) has been reported to be connected with various biological actions.[1] Recently, we found that vanadate stimulated the release of lipoprotein lipase, which hydrolized plasma triacylglycerides in very low density lipoprotein and chylomicron,[2] from isolated rat adipose tissue, and the increased enzyme activity in the tissue.[3, 4] The vanadate action may be partly dependent on the elevation of intracellular Ca^{2+} concentration.[3, 5] Mechanisms of the vanadate action in detail, however, are unknown. Inositol 1,4,5-trisphosphate (IP_3) is a well-known second messenger in a number of cell functions,[6, 7] such as the release of Ca^{2+} from non-mitochondrial Ca^{2+} pools.[8-10] We report here that vanadate rapidly increases the IP_3 content in the isolated adipose tissue.

The fat pads, which were prepared from rat epididymal adipose tissue as described previously,[3, 4] were incubated with various agents at 37 °C for indicated periods. The incubated fat pads were quickly frozen to terminate the reaction and homogenized in cold 12.5% trichloroacetic acid (TCA). After centrifugation at 16,000 X g for 10 min, the resultant supernatant was extracted with H_2O-saturated diethyl ether to remove TCA.[11] The obtained TCA-soluble fraction was subjected to the following assay of Ca^{2+}-release and the quantitative analysis of the IP_3 mass content using the commercially available IP_3 determination kit (Amersham, TRK. 1000; Tokyo). The uptake and release of Ca^{2+} from endoplasmic reticulum (ER) was assayed by the method of Delfert et al.[8] The ER preparation (500-900 ug) was loaded with $^{45}CaCl_2$ (81.4-133.2 KBq) in 0.5 ml of 50 mM Tris-Pipes buffer, pH 7.0, containing 0.2 M sucrose, 0.1 M KCl, 20 mM NaN_3, and 2 mM $MgCl_2$ at 30 °C, 20 min. The assay of the release of Ca^{2+} from $^{45}Ca^{2+}$-loaded ER (500 ug, 8.7 nmol Ca^{2+} / mg protein) was incubated with the TCA-soluble fraction in 0.5 ml of the buffer at 30 °C for the indicated periods. The incubation was terminated by a rapid filtration of 100 ul of the reaction mixture on a filter (Millipore, HA 0.45 um), followed by washing six times with 5 ml of 0.25 M sucrose. The filters were dried, and then the radioactivity was measured.

The results are mean ± SE of four or five observations for three separate experiments.

Figure 1 shows the time dependency of IP_3 content in the fat pads incubated with 2 mM vanadate over a 120-s period. A time-dependent increase was observed up to 30 s. The maximal increase in IP_3 content in the fat pads, which was two-fold higher than the basal level, was observed with a 30-s incubation. Insulin (15 nM) failed to significantly increase IP_3 content in fat pads under these experimental conditions .

Table I shows that the TCA-soluble fraction prepared from the vanadate-treated fat pads stimulates the release of Ca^{2+} with a 1-min incubation. No stimulatory release of Ca^{2+} was observed with the TCA-soluble fraction from insulin-treated fat pads.

Zick and Sagi-Eisenberg reported that a combination of vanadate and H_2O_2, but not vanadate alone, stimulated the protein phosphorylation and IP_3 formation in rat hepatoma cells during a 20-min incubation.[12] In contrast, Palmer et al. reported that the maximal IP_3 content in vasopressin-stimulated rat hepatocytes was observed with a short incubation period, for 5-15 s.[11]

Recently, Randazzo et al.[13] showed inositol phosphate accumulation in the presence of lithium and vanadate over a 60-min period. In our data, incubation of the fat pads with vanadate showed the maximal stimulatory IP_3 content at 30 s (Fig. 1), and the increase completely disappeared after 120 s.

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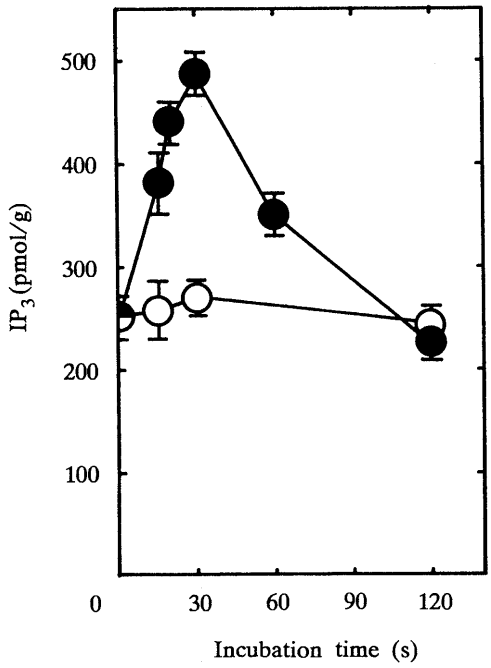

Fig. 1. Time course of IP_3 Content in Vanadate-Treated Adipose Tissue

The fat pads were incubated with vanadate (2 mM, ●), or insulin (15nM, ○) at 37°C for the indicated periods.

Table I. Effect of TCA-Soluble Fraction from Vanadate- or Insulin-Treated Fat Pads on Release of Ca^{2+} from ER

	Remaining $^{45}Ca^{2+}$ content in ER (%)		
Time after addition (min)	0	1	3
TCA-soluble fraction			
Vanadate (2 mM)-treated	100	73 ± 3.8	69 ± 5.4
Insulin (15 nM)-treated	100	105 ± 6.2	101 ± 4.0

The fat pads were incubated with vanadate (2 mM) or insulin (15 nM) at 37°C for 30 s. $^{45}Ca^{2+}$-loaded ER was incubated with each TCA-soluble fraction for indicated periods. The preparation of TCA-soluble fraction was described in the method for Fig. 1.

According to Delfert et al.,[8] IP_3 mobilized Ca^{2+} from the ER vesicle, but not from plasma membranes, and had no effect on Ca^{2+}-ATPase activity. In our experiment, the stimulatory release of Ca^{2+} from the ER by the TCA-soluble fraction of vanadate-treated fat pads may be independent of the change in Ca^{2+}-ATPase activity.

In conclusion, our results show that vanadate increases IP_3 content in the fat pads and probably consequently causes the elevation of intracellular level of Ca^{2+}.

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