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DEMONSTRATION OF RYANODINE-INDUCED METABOLIC EFFECTS IN RAT LIVER

Bethany Pereira, Li Feng, Roberto Bazotte*, Liliana Hernandez‡, Salvador Uribe‡ and Naomi Kraus-Friedmann††

Department of Physiology and Cell Biology, University of Texas Medical School at Houston, Houston, TX 77225, U.S.A.; and ‡Instituto de Fisiologia Cellular, Universidad National Autonomia de Mexico, 04510 Mexico, D.F.

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Abstract: The effects of ryanodine, a plant alkaloid which alters Ca^{2+} sequestration in the liver, on O_2 uptake and gluconeogenesis were measured. Ryanodine administration to perfused rat liver resulted in the stimulation of O_2 uptake and of gluconeogenesis. Because ryanodine does not affect directly mitochondrial respiration, its stimulatory effect on O_2 uptake in the whole cell is likely to be secondary to the increased cytosolic free Ca^{2+} levels.

Calcium is known to be involved in the regulation of many cellular processes. Changes in Ca^{2+} sequestration have been shown to be associated with and leading to numerous changes in cellular functions, e.g. muscle contraction and liver metabolism [1,2]. In studies on the role of Ca^{2+} in excitation-contraction coupling in muscle tissues, ryanodine, an insecticidal plant alkaloid, was employed successfully as an experimental tool [3-5]. Ryanodine affects muscle contraction by binding to a receptor which functions as a Ca^{2+} channel [4,5]. Interestingly, the most sensitive manifestation of the effect of ryanodine on muscle tissue is an increase in oxygen consumption [3]. It was reported recently that ryanodine dramatically stimulates respiration of frog sartorius muscle at a concentration of 10^{-11} M, when muscle contraction is not yet stimulated. This increase in respiration was attributed by the authors to an increase in Ca^{2+} uptake [6].

High-affinity ryanodine binding sites were described recently as present also in the rat liver [7-10]. However, the liver does not possess mRNA for the skeletal muscle ryanodine receptor and the binding sites do not interact with antibodies prepared against the purified skeletal or cardiac muscle receptors [8]. Thus, the hepatic binding sites are clearly different from the skeletal and cardiac muscle receptors and represent a different protein. However, binding of ryanodine to the hepatic receptor results also in increased cytosolic free Ca^{2+} level, [10]. Thus it might be that, like the muscle and brain receptors [11], the hepatic receptor also functions as a Ca^{2+} channel.

Because in the muscle O_2 uptake is the most sensitive indicator of ryanodine action, it was considered important to evaluate whether ryanodine administration also causes increases in O_2 consumption by the liver. The hepatic ryanodine receptor is a protein which is different from the skeletal and cardiac receptors; therefore, it is not obvious that the effects of ryanodine in the liver will be the same as in the muscle. Our results show that ryanodine also stimulates O_2 uptake in the liver but to a much lesser extent than in muscle tissues.

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*R. Bazotte's permanent address is: Department of Pharmacology, University of Maringa, Maringa, Brazil.

††Corresponding author: Dr. Naomi Kraus-Friedmann, Department of Physiology and Cell Biology, University of Texas Medical School, POB 20708, Houston, TX 77225. Tel. (713) 792-5278; FAX (713) 794-1349.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats, weighing 130-180 g, were used in all the experiments.

Materials. Ryanodine was obtained from Calbiochem, La Jolla, CA. Albumin, bovine (Cohn fraction V) was purchased from the Biochemical Corp., Cleveland, OH. Pyruvate, L-lactate, glucose oxidase (Trinder) reagent, and vasopressin were obtained from the Sigma Chemical Co., St. Louis, MO, and glucagon was from Eli Lilly & Co., Indianapolis, IN. All other reagents were of the highest obtainable purity.

Measurement of oxygen uptake. The uptake of oxygen in perfused liver was measured at room temperature (20°) essentially as described in an earlier study [12]. In isolated mitochondria O_2 uptake was measured as in previous studies [13,14].

Measurement of gluconeogenesis in the perfused liver. Livers from overnight fasted rats were perfused in situ at 32° as described previously [15].

Statistical analysis. The data were analyzed by the paired t-test using a computer program (primer Biostatistics: The Program).

RESULTS AND DISCUSSION

The effect of ryanodine on liver oxygen consumption is presented in Fig. 1. The effect of ryanodine on O_2 uptake seems to be immediate and evident as soon as the drug arrives to the liver. Because, structurally, ryanodine is a relatively large (17Å), very polar, and water-soluble molecule [16], it is hard to estimate exactly how much of the applied amount is actually entering into the cells.

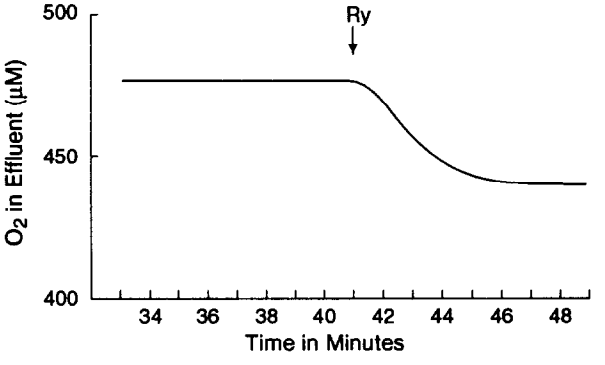

Fig. 1. Effect of ryanodine on liver O_2 uptake. Respiration was measured as described in Ref. 12. A fed rat was perfused for 30 min with regular KRB and 10 mM glucose. Then 2 μM ryanodine was added to the perfusate.

Ryanodine stimulates respiration in perfused livers from both fed and fasted rats. The rate of respiration before ryanodine was 2.09 ± 0.21 μatom O_2/min/g liver; after ryanodine: 2.30 ± 0.23 μatom O_2/min/g liver ($N = 6$; $P = < 0.001$). The effects of two hormones known to increase oxygen consumption in the liver were also measured (results not shown). The increase in respiration obtained with maximally effective doses of either glucagon or vasopressin is in the same range as the increase observed with ryanodine. The observed increases in the rate of respiration which followed ryanodine administration in the liver are less than the increases observed in either insects or in vertebrate muscle preparations where several-fold increases have been reported [3,6]. The stimulation in the liver amounts to about 20% of the unstimulated rate. It is relevant to point out that ryanodine-binding sites were recently identified in the hepatic mitochondrial fraction [9]. Therefore, the possibility that ryanodine stimulates respiration directly through these binding sites needed to be examined. The results, shown in Table 1, demonstrate that ryanodine added directly to isolated mitochondria had no effect on O_2 uptake. Therefore, it is likely that the increase in respiration observed in

the liver is secondary to the increases in cytosolic free Ca^{2+} and can be attributed to the stimulation of intramitochondrial dehydrogenases [17] as is the case in muscle [6,18].

Table 1. Effects of ryanodine on O_2 consumption by isolated rat liver mitochondria

	QO_2 rates (natom O_2/min/mg protein)		
	Ryanodine concentration (μM)		
	0	2	5
State 4	17 ± 3	20 ± 2	18 ± 4
State 3	88 ± 13	91 ± 8	87 ± 8
RC (3/4)	5.18	4.55	4.83

Values are means ± SD; N = 3.

It has been pointed out, based on earlier studies, that a redistribution of Ca^{2+} and stimulation of respiration are characteristics of hormonal stimulation of gluconeogenesis [19]. Because these were the effects evoked by ryanodine in the liver, namely, increased cytosolic free Ca^{2+} and stimulated rate of respiration, it seemed appropriate to check whether or not ryanodine also increases gluconeogenesis in the liver. In Fig. 2, the results of these experiments are presented.

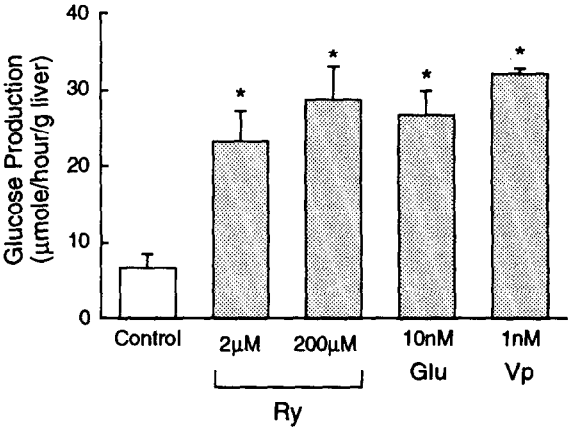

Fig. 2. Effects of ryanodine and hormones on gluconeogenesis in the perfused liver. Glucose production was measured as described in Ref. 15. Results are the means ± SEM of 3-6 experiments. Key: (*) significantly different from control ($P < 0.01$).

Ryanodine increased the rate of glucose production in the perfused liver to the same level observed in the present experiments in the presence of maximally effective concentrations of glucagon and vasopressin. Ryanodine also stimulated gluconeogenesis by 36% in the isolated hepatocyte preparation (results not shown). Because ryanodine has no other known effect than altering Ca^{2+} sequestration, the stimulatory effect of ryanodine on gluconeogenesis reinforces the postulate that this effect is sufficient for, and results in, an increased rate of glucose production by the liver in the fasted state. An example of this mechanism is the short-term stimulation of gluconeogenesis by thyroid hormone, which acts primarily by changing Ca^{2+} sequestration and stimulating respiration [20,21], or the stimulation of gluconeogenesis by extracellular ATP [22].

In summary, data are presented which demonstrate that ryanodine administration profoundly affects liver metabolism. It seems likely that these metabolic effects of ryanodine are the consequence of the alterations

in Ca^{2+} sequestration observed after ryanodine administration. Thus, ryanodine can serve as a useful tool to explore Ca^{2+}-dependent processes in the liver.

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