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# Adenine Nucleotide Pattern in Rat Pancreatic Islets Exposed to Nutrient Secretagogues

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**The effects of D-glucose, D-mannose, D-galactose, D-glyceraldehyde, pyruvate, L-lactate, 2-ketoisocaproate, L-leucine, and/or L-glutamine on the ATP and ADP content of rat isolated pancreatic islets were reevaluated in order to compare changes evoked by these nutrient secretagogues in the islet ATP content and ATP/ADP ratio to their effects upon insulin release. Although being compatible with the fuel concept for nutrient-stimulated insulin secretion, the results of this study also argue against the monolithic view that the adenine nucleotide pattern in islet cells represents the sole coupling factor between metabolic and more distal events in the process of nutrient-stimulated insulin release.**

**Key Words:** Pancreatic islets; ATP and ADP content; insulin release; nutrient secretagogues.

## Introduction

When the fuel hypothesis for nutrient-stimulated insulin release *(1)* was validated as a fuel concept *(2)*, the proposal was made that the coupling between metabolic events and more distal ionic and secretory events in the insulin-producing islet B-cells represents a multifactorial process involving changes in the generation of ATP, reducing equivalents [NAD(P)H], and protons ($H^+$) *(3)*. Soon thereafter, however, emphasis was placed on the role of cytosolic ATP in regulating the activity of ATP-sensitive $K^+$ channels located at the plasma membrane *(4)*. Thus, the closing of such channels in response to an increase in cytosolic ATP concentration was postulated to lead to depolarization of the plasma membrane, subsequent gating of voltage-sensitive $Ca^{2+}$ channels, and eventual activation by the divalent cation of the microtubular–microfilamentous system controlling the access of secretory granules to exocytotic sites *(5)*.

More recently, such a model was adapted to take into account on ATP-dependent modality of insulin release not linked to the closing of ATP-sensitive $K^+$ channels *(6)*. Moreover, renewed interest was focused on the finding first reported by Carpinelli and Malaisse *(7)* that the relationship between $K^+$ conductance, as judged from the efflux of $^{86}Rb^+$ from prelabeled islets, and extracellular D-glucose concentration, while accounting for the maintenance of a low insulin output at hexose concentrations below the threshold value for stimulation of insulin secretion, was apparently not adequate to account for the insulinotropic action of D-glucose at higher concentrations. Hence, attention was drawn to the possible participation of other changes in ionic fluxes, e.g., the gating of volume-sensitive anion channels, in the stimulus–secretion coupling for nutrient-induced insulin release *(8,9)*.

In the light of these considerations, the major aim of the present study was to compare the effects of 10 distinct nutrients or combination of nutrients upon the ATP and ADP content of isolated rat islets, all nutrients being tested at the same 10 m*M* concentration. The selection of this research theme was also motivated by the recent observation of a paradoxical decrease in ATP content and ATP/ADP ratio in islets exposed to D-fructose *(10)*.

## Results

D-Glucose (10 m*M*) increased ($p < 0.001$) the ATP content, total ATP + ADP content, and ATP/ADP ratio above the basal values found in islets incubated in the absence of any exogenous nutrient (Table 1).

Likewise, D-mannose (10 m*M*) increased ($p < 0.005$) the ATP content and ATP/ADP ratio above the corresponding basal values found within the same experiments. The increase in the total ATP + ADP content caused by D-mannose was also significant ($p < 0.05$). None of the four variables under consideration was significantly different in islets exposed to either D-glucose or D-mannose.

D-Galactose (also 10 m*M*) failed to affect significantly any of these four variables, when comparing the data obtained within the same experiments in the presence vs the absence of this hexose. The values for the ATP content and ATP/ADP ratio found in the presence of D-galactose remained much lower ($p < 0.01$ or less) than those recorded, within the same experiments, in the presence of D-glucose.

D-Glyceraldehyde (again 10 m*M*) augmented ($p < 0.025$) the ATP/ADP ratio above basal value. Its effect to increase

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**Table 1**
Adenine Nucleotide Content of Islets Exposed to Nutrient Secretagogues

| Exp. No. | Nutrient (10 m*M*) | ATP (pmol/islet) | ADP (pmol/islet) | ATP + ADP (pmol/islet) | ATP/ADP (ratio) |
|---|---|---|---|---|---|
| 1 | Nil | 3.81 ± 0.44 (18) | 3.05 ± 0.92 (11) | 6.08 ± 0.92 (11) | 0.965 ± 0.061 (11) |
| | D-glucose | 6.25 ± 0.54 (17) | 3.40 ± 0.48 (10) | 8.77 ± 1.10 (10) | 1.649 ± 0.171 (10) |
| | D-mannose | 5.95 ± 0.48 (18) | 3.69 ± 0.47 (10) | 8.80 ± 0.32 (10) | 1.681 ± 0.392 (10) |
| | D-galactose | 4.35 ± 0.44 (18) | 3.49 ± 0.46 (12) | 7.21 ± 0.78 (12) | 1.078 ± 0.098 (12) |
| | D-glyceraldehyde | 4.42 ± 0.31 (18) | 3.30 ± 0.33 (12) | 7.43 ± 0.55 (12) | 1.377 ± 0.153 (12) |
| 2 | Nil | 4.22 ± 0.35 (14) | 3.79 ± 0.29 (13) | 7.84 ± 0.58 (13) | 1.082 ± 0.050 (13) |
| | D-glucose | 6.12 ± 0.53 (17) | 4.16 ± 0.32 (17) | 10.28 ± 0.75 (17) | 1.528 ± 0.130 (17) |
| | Pyruvate | 4.46 ± 0.43 (17) | 3.55 ± 0.30 (17) | 7.94 ± 0.56 (17) | 1.314 ± 0.140 (17) |
| | L-lactate | 3.91 ± 0.32 (17) | 3.00 ± 0.12 (17) | 6.91 ± 0.35 (17) | 1.327 ± 0.113 (17) |
| | 2-Ketoisocaproate | 6.05 ± 0.59 (18) | 4.09 ± 0.20 (18) | 10.14 ± 0.71 (18) | 1.479 ± 0.125 (18) |
| 3 | Nil | 4.33 ± 0.66 (17) | 4.04 ± 0.56 (17) | 8.37 ± 1.19 (17) | 1.060 ± 0.040 (17) |
| | D-glucose | 7.03 ± 0.73 (16) | 4.97 ± 0.51 (16) | 12.00 ± 1.15 (16) | 1.460 ± 0.130 (16) |
| | L-leucine | 7.14 ± 0.86 (17) | 4.04 ± 0.34 (17) | 11.17 ± 1.09 (17) | 1.801 ± 0.235 (17) |
| | L-glutamine | 4.50 ± 0.48 (18) | 2.94 ± 0.21 (17) | 7.39 ± 0.61 (17) | 1.592 ± 0.184 (17) |
| | L-leucine + L-glutamine | 6.85 ± 0.73 (16) | 3.96 ± 0.32 (16) | 10.81 ± 0.91 (16) | 1.802 ± 0.168 (16) |

the ATP content was of doubtful statistical significance ($p < 0.07$). The ATP content of the islets exposed to D-glyceraldehyde was lower ($p < 0.005$) than that found, within the same experiments, in islets incubated in the presence of D-glucose. The other variables were not significantly different, however, under these two experimental conditions.

Pyruvate (10 m*M*) failed to affect significantly any of the four variables relative to the values found, within the same experiments, in islets deprived of exogenous nutrient. Nevertheless, the ATP/ADP ratio in the islets exposed to pyruvate yielded a mean value (1.31 ± 0.14; $n = 17$) halfway between that found in islets not exposed to any exogenous nutrient (1.08 ± 0.05; $n = 13$) and those exposed to D-glucose (1.53 ± 0.13; $n = 17$). While both the ATP content and total ATP + ADP content were significantly lower ($p < 0.01$ or less) in the islets incubated in the presence of pyruvate as compared to D-glucose, the ATP/ADP ratio was not significantly different ($p > 0.1$) under these two experimental conditions.

The situation found in islets exposed to L-lactate (10 m*M*) was virtually identical to that found in islets exposed to pyruvate (also 10 m*M*). Thus, lactate failed to affect significantly any of the variables under consideration relative to the corresponding basal values, but again yielded an ATP/ADP ratio (1.33 ± 0.11; $n = 17$) in between that found, within the same experiments, under basal conditions (1.08 ± 0.05; $n = 13$) and in the presence of D-glucose (1.53 ± 0.13; $n = 17$). Moreover, while the ATP content, ADP content, and ATP + ADP total content all remained significantly lower ($p < 0.005$ or less) in islets exposed to lactate than the corresponding values found within the same experiments in islets exposed to D-glucose, such was not the case ($p > 0.1$) for the ATP/ADP ratio.

The situation prevailing in islets exposed to 2-ketoisocaproate (10 m*M*) was virtually identical to that found, within the same experiments, in islets exposed to D-glucose (also 10 m*M*). The 2-keto acid indeed augmented significantly ($p < 0.02$ or less) the ATP content, ATP + ADP total content, and ATP/ADP ratio, but not the ADP content, above basal values. None of these four variables was significantly different in islets exposed to either 2-ketoisocaproate or D-glucose.

Likewise, L-leucine (10 m*M*) augmented significantly ($p < 0.005$ or less) the ATP content, ATP + ADP total content, and ATP/ADP ratio, but not the ADP content, above basal values. None of the four variables was significantly different in islets exposed to either L-leucine or D-glucose.

Like pyruvate and L-lactate, L-glutamine (10 m*M*) failed to affect significantly the ATP, ADP, or total ATP + ADP content, relative to the basal values found within the same experiments, but increased significantly ($p < 0.01$) the ATP/ADP ratio.

In the concomitant presence of L-leucine and L-glutamine (10 m*M*), the results were virtually identical to those recorded, within the same experiments, in the sole presence of the branched chained amino acid. In both cases, none of the four variables were significantly different from those recorded, again within the same experiments, in the presence of D-glucose.

For the sake of comparison between the metabolic data listed in Table 1 and the corresponding insulinotropic action of the nutrients under consideration, Table 2 provides information on the release of insulin recorded under similar experimental conditions as those used in the measurements of adenine nucleotides.

## Discussion

The present results indicate that, with the sole exception of D-galactose, which fails to stimulate insulin release *(11)*, all nutrients explored in this study augmented the ATP/ADP

**Table 2**
Insulin Release by Islets Exposed to Nutrient Secretagogues

| Nutrient[a] | Insulin output | Reference(s) |
|---|---|---|
| Nil | 10.6 ± 3.6 (36)[b] | *28* |
| D-glucose | 100.0 ± 4.8 (36) | *28* |
| D-mannose | 34.0 ± 7.7 (10) | *29* |
| D-galactose | 9.6 ± 1.3 (23) | *11* |
| D-glyceraldehyde | 54.3 ± 4.3 (15) | *30* |
| Pyruvate | 11.8 ± 3.1 (28) | *12* |
| L-lactate | 12.5 ± 2.6 (44) | *13* |
| 2-Ketoisocaproate | 73.2 ± 2.8 (70) | *22* |
| L-leucine | 38.5 ± 1.8 (74) | *22,31* |
| L-glutamine | 9.4 ± 1.5 (40) | *31* |
| L-leucine + L-glutamine | 139.8 ± 9.3 (28) | *31* |

[a]The nutrient concentration corresponds to that mentioned in Table 1.

[b]All results are expressed relative to those recorded in the presence of D-glucose.

ratio above the basal value found in the absence of exogenous nutrient. Such an increase was more modest in the presence of pyruvate, L-lactate, and L-glutamine, than in that of D-glucose, D-mannose, 2-ketoisocaproate or L-leucine.

For instance, the normalized ATP/ADP ratio found in the islets exposed to pyruvate and L-lactate (101.1 ± 5.4%; $n = 34$) was significantly higher ($p < 0.001$) than basal value (82.4 ± 2.2%; $n = 41$), but significantly lower ($p < 0.025$) than that found in the islets exposed to D-glucose (119.1 ± 5.6%; $n = 43$).

Pyruvate, L-lactate, and L-glutamine also shared the property of failing to affect significantly the ATP content, ADP content, and total ATP + ADP content of the islets. These three nutrients, which do not significantly enhance insulin output above basal value, are nevertheless able to augment insulin secretion in the presence of other nutrients or environmental factors *(12–15)*. In other words and at variance with D-galactose, they display, under suitable experimental conditions, a sizable insulinotropic efficiency.

D-glucose, D-mannose, 2-ketoisocaproate, and L-leucine not only augmented the ATP/ADP ratio but also increased significantly the ATP content and total ATP + ADP content above the corresponding basal values. These four nutrients are able to stimulate insulin release, albeit to a variable extent, even in the absence of any other insulin secretagogues *(16,17)*.

The situation found here with D-glyceraldehyde was somewhat different from that encountered with the other nutrients explored in this study. Thus, on the one hand, the triose slightly augmented the islet ATP content above basal value. Such an effect only reached statistical significance ($p < 0.03$), however, by comparison with the mean normalized value derived from all determinations made in islets deprived of exogenous nutrient. This was also the case ($p < 0.05$) when considering the effect of the triose upon the total ATP + ADP islet content. Even so, the ATP content of islets incubated in the presence of D-glyceraldehyde remained significantly lower than that found in islets exposed to D-glucose, whether such a difference was judged from data recorded within the same experiment(s) ($p < 0.005$) or from the comparison between all available normalized values ($p < 0.02$). On the other hand, even according to the latter analytical procedure, the ATP/ADP ratio found in islets exposed to D-glyceraldehyde, which stimulates insulin secretion in the absence of any other insulinotropic agent *(18)*, was not significantly lower ($p > 0.25$) from that found in islets exposed to D-glucose.

At this point, it should be emphasized, that the present data were, as a rule, in good agreement with prior findings, whenever already available *(12–14,16–21)*.

From the results so far considered in this discussion, the impression could be gained that there was a close analogy between the effects of the various nutrients examined in this study upon the adenine nucleotide profile and insulin secretory activity of isolated islets. The following exceptions to such a rule should not be ignored, however.

First, no difference could be detected between islets exposed to D-glucose and D-mannose, although the secretory response to the latter hexose is lower than that evoked by the former hexose, especially at concentrations close to those tested in the present study *(20)*.

Second, as judged from results obtained in separate experiments, the mean ATP/ADP ratio was lower, albeit not significantly so ($p > 0.15$ or more), in islets exposed to 2-ketoisocaproate than in those exposed to L-leucine, the former results averaging 82.1 ± 6.9 and 78.3 ± 6.2 % ($n = 18$ in both cases) of the latter ones, as judged from the absolute and normalized values, respectively. Yet the release of insulin evoked by 2-ketoisocaproate is much higher than that evoked by L-leucine *(22)*. This second example of an apparent dissociation between adenine nucleotide and insulin output points toward the participation of other factors than changes in ATP metabolism in the process of nutrient-stimulated insulin release. And indeed, in a prior report, emphasis was placed on the unfavorable effect of $NH4^+$ accumulation in the islets in the process of leucine-stimulated insulin secretion *(23)*.

The third most obvious discrepancy between changes in islet ATP and ADP content and insulin output refers to the effect of L-glutamine in islets exposed to L-leucine. Thus, as judged from both the present and prior results, the ATP content, ADP content, total ATP + ADP content, and ATP/ADP ratio are virtually identical in islets exposed to L-leucine alone and in those exposed to both L-leucine and L-glutamine *(21)*. Yet, L-glutamine dramatically augments the insulin secretory response to L-leucine *(21)*.

As a first step to elucidate the determinants responsible for these dissociated metabolic vs secretory responses, it

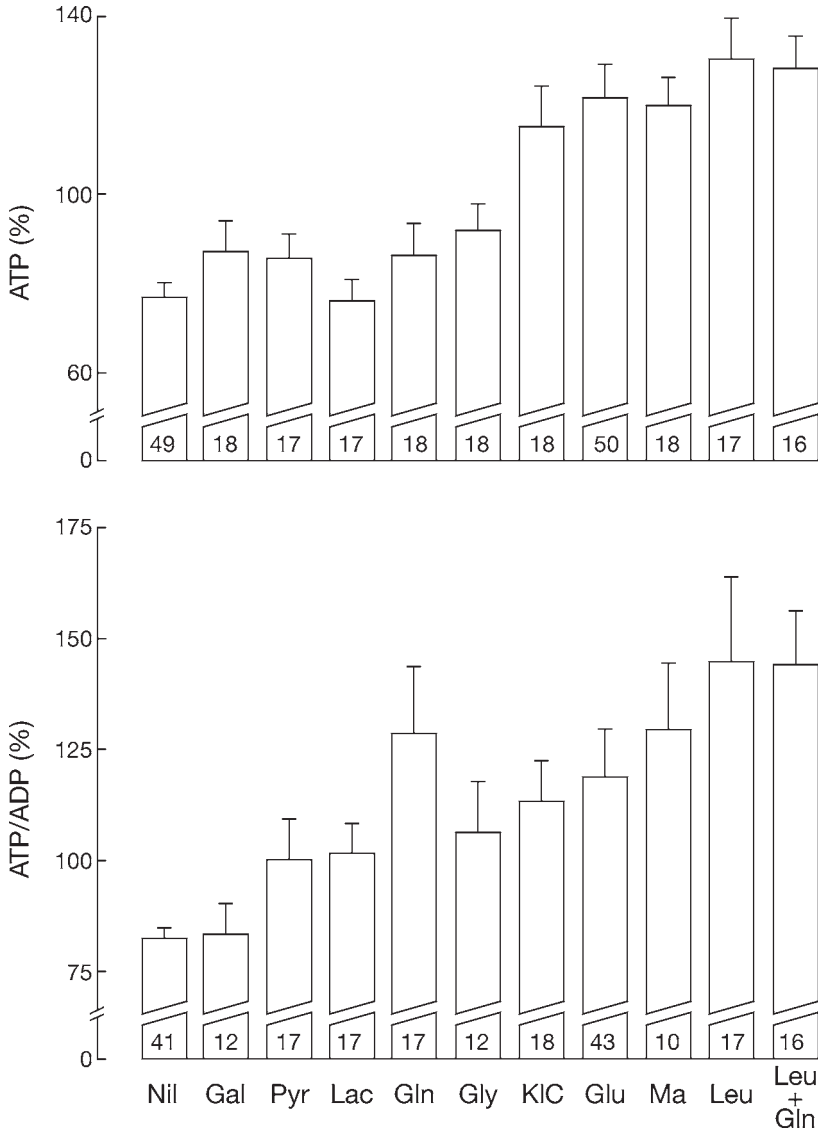


**Fig. 1.** Normalized values (percent) for the ATP content (upper panel) and ATP/ADP ratio in islets deprived of exogenous nutrient (Nil) or exposed to D-galactose (Gal), pyruvate (Pyr), L-lactate (Lac), L-glutamine (Gln), D-glyceraldehyde (Gly), 2-ketoisocaproate (KIC), D-glucose (Glu), D-mannose (Ma), L-leucine (Leu), or both L-leucine and L-glutamine (Leu/Gln), all tested at a 10 m*M* concentration. Mean values (± SEM) refer to the number of separate determinations indicated at the bottom of each column.

may be desirable to compare the present results to measurements of ATP and ADP content made in the cytosolic compartment of islet cells. Indeed, this approach recently allowed metabolic and secretory data collected in islets exposed to D-fructose to be reconciled *(10)*. Further work is presently in progress to provide such a comparison in the case of the nutrients tested in the present study. It should indeed be kept in mind that the regulation of events located at the plasma membrane, e.g., the closing of ATP-sensitive $K^+$ channels, is dependent on the cytosolic concentration of such factors as ATP and ADP.

Meanwhile, the present data, while providing further support to a key role for ATP generation in the insulinotropic action of selected nutrients, may well pave the way to a more nuanced interpretation of the multifactorial coupling process between metabolic and secretory events in insulin-producing islet cells stimulated by nutrient secretagogues.

## Materials and Methods

All experiments were conducted in pancreatic islets prepared by the collagenase method from fed female Wistar rats *(24)*.

Batches of 12 freshly isolated islets each were incubated for 60 min at 37°C in 0.36 mL of a bicarbonate- and HEPES-buffered salt-balanced medium *(25)* containing bovine serum albumin (5 mg/mL) and the tested agent(s) and equilibrated against a mixture of $O_2/CO_2$ (19/1, v/v). The incubation was halted and extraction procedure of adenine nucleotides

was achieved by addition of 0.12 mL of a solution of NaOH (160 m*M*) containing 0.4 m*M* ammonium monovanadate (ATPase inhibitor), 6.0 m*M* EDTA, and Triton X-100 (0.0004%, w/v). After 10 min incubation at 85°C, in order to denature the enzymes and protect adenine nucleotides from degradation, and 5 min centrifugation at 20°C and 1000*g*, the supernatant was sonicated (three times for 10 s) on ice and neutralized by addition of 30 µL of HCl (650 m*M*). Then, 0.12 mL of an imidazole buffer (262 m*M*, pH 7.00) containing KCl (394 m*M*) and MgCl2 (10 m*M*) was added to all tubes (islet samples, control media without islet, ATP, and ADP standards) and in each of them two aliquots (0.27 mL each) were taken and frozen until ATP assay. On the day of this assay (see below) one of the two aliquots was mixed with 30 µL of an imidazole buffer (100 m*M*, pH 7.75) while the other was mixed with 30 µL of the same buffer also containing 3.0 m*M* phospho-enol-pyruvate (Roche Diagnostics) and 0.02 mg of rabbit muscle pyruvate kinase (E.C. 2.7.1.40; 200 units/mg at 25°C; Roche Diagnostics) to convert ADP into ATP. After 30 min incubation at 30°C, the reaction was halted by heating for 10 min at 85°C. The samples were cooled, centrifuged for 1 min in Beckman microfuge, and ATP was assayed on aliquot portions (100 µL each) by a luminometric method *(26,27)*.

In each experiment, four to six groups of islets were incubated in the absence of exogenous nutrient, in the presence of D-glucose (10 m*M*), and in the presence of three other nutrients (or combination of two nutrients). Three such experiments were conducted under identical experimental conditions, with three series of experimental conditions under investigation. The absolute values recorded in each of these three series (Exp. Nos. 1, 2, and 3) are given in Table 1. The results obtained in each experiment were also normalized relative to the mean of the mean values found in the absence of exogenous nutrient and presence of D-glucose in that same experiment. Such normalized values were then eventually pooled together for each tested nutrient(s), the results being illustrated in Fig. 1.

All results are expressed as mean values (±SEM), together with the number of separate determinations (in parentheses). The statistical significance of differences between mean values was assessed by use of Student's *t*-test.

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