Measurement of Free Magnesium in Perfused and Ischemic Arrested Heart Muscle. A Quantitative Phosphorus-31 Nuclear Magnetic Resonance and Multiequilibria Analysis†

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ABSTRACT: The concentration of intracellular free Mg^{2+} in perfused, beating guinea pig hearts has been estimated on the basis of the Mg^{2+}-dependent separation between the α- and β-phosphorus resonances from ^{31}P NMR spectra of intracellular ATP combined with a computer-assisted analysis of the multiequilibria involved. Measurements and calculations are also used to determine the magnitude of any change in free Mg^{2+} concentration which may occur during the severe acidosis associated with ischemic arrest. We find the concentration of free Mg^{2+} to be 2.5 ± 0.7 mM in control hearts. No significant change was observed during ischemic arrest. These results are discussed in relation to other similar measurements in the literature with detailed consideration given to the value of the MgATP stability constant.

Measurement of the concentration of free Mg^{2+} in perfused beating heart preparations has been an important but relatively intractable problem. Considerations of the multiequilibria involved and the virtual absence of acceptable methods for exact measurements within the cytosol under physiologic conditions have hampered progress (Polimeni & Page, 1973). Recently, a direct measure of the activity of free Mg^{2+} in living tissue has become feasible through the application of phosphorus NMR to biological cells and perfused organs [see Shulman et al. (1979) for a recent review]. The measurement is based on the early demonstration of Cohn & Hughes (1962) that the spectrum of ATP was sensitive to metal ion chelation. Hoult et al. (1974) and Burt et al. (1976) showed that ≥95% of the cytosolic ATP in resting skeletal muscle was in the Mg^{2+}-bound form. Based on this demonstration, one can calculate the concentration of free Mg^{2+} by knowing the stability constant, total ATP concentration, and the fraction of ATP complexed (Gupta et al., 1978; Gupta & Yushok, 1980; Gupta & Moore, 1980). Despite the distinct advances which NMR studies have offered to cell and organ physiology, there has been no quantitatively satisfying explanation for the poor agreement between the value of free Mg^{2+} concentration obtained from theoretical calculations (3.37 mM) (Nanninga, 1961) and the value obtained from NMR measurements (0.6 mM) (Gupta & Moore, 1980) for the same type of tissue (i.e., frog skeletal muscle).

The purpose of the present study was to determine the concentration of free Mg^{2+} in perfused, beating guinea pig hearts by using ^{31}P NMR chemical-shift differences between the α- and β-phosphorus resonances of intracellular ATP. The calculations based on the NMR measurements were then compared with computer-assisted calculations, considering all significant equilibria involved. With this dual approach, we then studied the effect of ischemic arrest on free Mg^{2+} concentration levels by considering the corresponding changes in pH and ATP, phosphocreatine (PCr), and P_i concentrations. Theoretical calculations are once again compared to calculations of free Mg^{2+} concentration based on the chemical-shift measurements between the α- and β-phosphorus resonances of ATP for the same hearts. The central consideration for calculations based on NMR chemical shifts is the true value of the MgATP association constant. This problem is extensively reviewed and discussed.

Materials and Methods

Male Hartley strain guinea pigs (450–550 g) were injected intraperitoneally with 3000 units of heparin at 20 min prior to anesthetization with 100 mg/kg of 5% thiamylal sodium (Surital, Parke, Davis and Co., Detroit, MI). Each heart was rapidly excised and arrested in ice-cold perfusion medium. Hearts were then mounted and secured on a Teflon cannula and perfused in a nonrecirculating Langendorff mode with modified Krebs–Henseleit bicarbonate buffer (pH 7.4) containing 5.5 mM glucose (Salhany et al., 1979). For the Mg^{2+} studies, perfusion was switched 2 min prior to termination of the perfusion to a Mg^{2+}-free buffer in order to flush extracellular spaces of perfusate Mg^{2+} (Alto & Dhalla, 1979).

^{31}P NMR spectra were collected at 60.7 MHz with a Nicolet NTC 150 spectrometer (Nicolet Instrument Corp., Mountain View, CA) operating in the Fourier-transform mode. Spectra were collected with 8K of memory from a 2.0-kHz sweep width at 1-s cycle time. After an initial 10-min equilibrium perfusion period, cannulated hearts were inserted in a 2.0-cm Teflon-stoppered NMR tube and placed in the magnetic field. Control spectra and subsequent sequential spectra represent 480 pulses with a total acquisition time of 10 min. Following acquisition of the control spectrum, the bathing medium was removed from the NMR tube. Ischemic arrest was then produced by cross-clamping the aortic perfusate inflow line. It was found that transient removal of the bathing medium facilitated a more rapid and complete normothermic arrest. Two minutes later, the bathing buffer was reintroduced into the NMR tube. Sequential NMR spectra were then collected as just described.

Hearts were frozen for metabolic determinations between blocks of Wollenberger tongs and cooled in liquid nitrogen. A 150–250-mg portion of the frozen tissue was weighed and dried to constant weight at 95 °C in pretared beakers to determine the dry to wet tissue weight ratio. Another portion of the tissue was weighed and pulverized with 6% perchloric

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Table I: Association Constants for the Various Equilibria under Consideration

reaction	K (M^{-1})	ref
$H\text{-}ATP^{3-} + H^+ \longleftrightarrow H_2ATP^{2-}$	$K_{H_2ATP} = 8.5 \times 10^3$	*a*
$ATP^{4-} + H^+ \longleftrightarrow H\text{-}ATP^{3-}$	$K_{H\text{-}ATP} = 1.09 \times 10^7$	*b, c*
$ATP^{4-} + K^+ \longleftrightarrow K\text{-}ATP^{3-}$	$K_{K\text{-}ATP} = 14$	*a*
$H\text{-}ATP^{3-} + Mg^{2+} \longleftrightarrow MgH\text{-}ATP^{1-}$	$K_{MgH\text{-}ATP} = 5.42 \times 10^2$	*b, c*
$ATP^{4-} + Mg^{2+} \longleftrightarrow MgATP^{2-}$	$K_{MgATP} = 3.48 \times 10^4$	*b, c*
$MgATP^{2-} + Mg^{2+} \longleftrightarrow Mg_2ATP$	$K_{Mg_2ATP} = 40$	*d*
$Cl^- + Mg^{2+} \longleftrightarrow MgCl^+$	$K_{MgCl} = 3.4$	*e*
$PCr + Mg^{2+} \longleftrightarrow MgPCr$	$K_{MgPCr} = 40$	*f*
$myosin + Mg^{2+} \longleftrightarrow Mg\text{-}myosin$	$K_{Mg\text{-}myosin} = 4 \times 10^2$	*g*
$sugar\text{-}P + Mg^{2+} \longleftrightarrow Mg\text{-}sugar\text{-}P$	$K_{Mg\text{-}sugar\text{-}P} = 50$	*f*
$HPO_4^{2-} + Mg^{2+} \longleftrightarrow MgHPO_4$	$K_{MgHP} = 40$	*h*
$myosin + K^+ \longleftrightarrow myosin\text{-}K$	$K_{K\text{-}myosin} = 1 \times 10^2$	*i*
$HPO_4^{2-} + H^+ \longleftrightarrow H_2PO_4^{1-}$	$K_{H_2P} = 5.4 \times 10^6$	*j*
$sugar\text{-}P + H^+ \longleftrightarrow sugar(HP)$	$K_{sugar(HP)} = 5.8 \times 10^6$	*f*

[a] O'Sullivan & Perrin (1964). [b] Phillips et al. (1966). [c] Storer & Cornish-Bowden (1976). [d] Noat et al. (1970). [e] Blair (1970). [f] *Stability Constants* (1971). [g] Nanninga (1957). [h] Holt et al. (1954). [i] Lewis & Saroff (1957). [j] Determined in this laboratory.

acid in a liquid nitrogen chilled stainless-steel percussion mortar. Neutralized extracts were taken for spectrophotometric analysis of ATP and phosphocreatine by utilizing a coupled enzymatic reaction (Lamprecht & Stein, 1965; Lamprecht & Trautschold, 1965). Magnesium ion analysis was performed by atomic absorption spectroscopy. Approximately 50 mg of frozen tissue was weighed and digested in concentrated HNO_3. Extracts for Mg^{2+} determinations were diluted 1:100 with La_2O_3 (dissolved in HCl).

Computer analysis was performed with a program capable of iterative calculations (Storer & Cornish-Bowden, 1976). The results presented in the text and tables are expressed as means ± standard deviation unless otherwise indicated.

The stability constant for MgATP was measured exactly as described by Burton (1959) except that we used 8-hydroxyquinoline-5-sulfonic acid (Aldrich, Milwaukee, WI). Otherwise, the conditions were pH 7.1, 25 °C, in 25 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (Hepes) buffer containing 0.15 M KCl. The measurements were made with a Gilford 250 recording spectrophotometer. The concentration of ATP used was directly measured for each run by using the enzymatic reaction of Lamprecht & Trautschold (1965). Total Mg^{2+} concentration was also directly measured for each run by using atomic absorption spectroscopy. Adolfsen & Moudrianakis (1978) have compared the dye method of Burton (1959) with a divalent cation electrode method. Although the dye method is subject to certain errors when used at high pH values, these errors seem minimal at the pH we use.

Results and Discussion

The estimation of free intracellular Mg^{2+} levels in intact muscle by using the phosphorus NMR spectrum of ATP has been thoroughly discussed by Gupta & Moore (1980). The method requires measurement of the chemical shift between the α and β resonances followed by calculations based on the stability constant for the interaction. Since the value of the stability constant is central to the calculation, it is necessary either to know the stability constant free of other interactions and then include all other significant interactions in the calculation or to measure the stability constant under simulated intracellular conditions and then use the experimentally determined value. Our approach to the problem was to use both intrinsic constants (Table I), considering all relevant equilibria, and to directly measure the stability constant under conditions similar to those used by Gupta & Moore (1980).

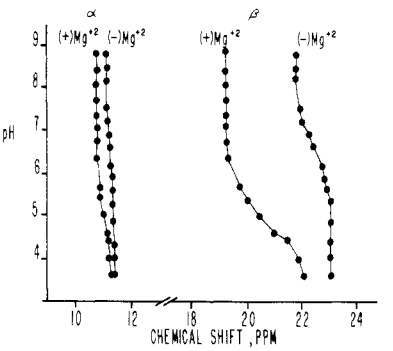

FIGURE 1: pH dependence of the α- and β-phosphorus resonance of ATP in the presence and absence of Mg^{2+}. The spectra were measured on 5 mM ATP solutions containing 100 mM KCl, 1 mM EDTA, and 10 mM [bis(2-hydroxyethyl)amino]tris(hydroxymethyl)methane (Bis-Tris) buffer with 10 mM or without $MgCl_2$ at 25 °C.

We consider first the effect of pH variations on the concentration of species and therefore the calculation of an apparent stability constant at pH 7 (i.e., intracellular cardiac pH; Salhany et al., 1979). The effects of pH variation on measured chemical shifts of the α- and β-phosphorus resonances are shown in Figure 1. It seems apparent from this figure that there is a significant titration of the β-ATP resonance at pH 7 in the absence of Mg^{2+} with an apparent pK shift in the presence of Mg^{2+}. Thus, quite significant levels of $H\text{-}ATP^{3-}$ species should exist at pH 7. This point is made particularly clear by the computer-assisted calculations shown in Table II based on the intrinsic constants listed in Table I. As can be seen, there are quite significant levels of $K\text{-}ATP^{3-}$ and $H\text{-}ATP^{3-}$ as compared with ATP^{4-} at pH 6 and 7.0 in the presence of Mg^{2+}. These species need to be taken into consideration when calculating the apparent stability constant for Mg^{2+} binding to ATP.

An expression for K', the apparent association constant, can be obtained by using the constants of Table I and equations developed by Gupta & Moore (1980). The equation is

$$K' = K_{MgATP}/[1 + (H^+)K_{H\text{-}ATP} + (K^+)K_{K\text{-}ATP}] \quad (1)$$

The ratio $(ATP)_T/(ATP)_f$ can be found by equating the measured relative separation between the α- and β-phosphorus resonances of the NMR spectrum with the weighted summation of corresponding separations of ATP standard solutions according to expressions given in Gupta & Moore (1980).

In order to see how the concentration of free intracellular Mg^{2+} should vary with total cytosolic Mg^{2+} concentration, we

Table II: ATP and Mg Species Present in Mixtures of ATP, K^+, and Mg^{2+} at pH 6 and 7 [a]

pH	(ATP^{4-})	($H\text{-}ATP^{3-}$)	(H_2ATP^{2-})	($K\text{-}ATP^{3-}$)	($MgATP^{2-}$)	($MgH\text{-}ATP^{1-}$)	(Mg_2ATP)	(Mg^{2+})	($MgCl^+$)
7.0	0.05	0.05		0.10	6.61	0.11	1.08	4.07	2.05
6.0	0.04	0.40		0.08	5.57	0.95	0.97	4.36	2.19
7.0	1.94	2.11		3.96					
6.0	0.57	6.20	0.05	1.18					

[a] The concentration of all species, expressed in millimolar, were calculated for a mixture containing 8 mM ATP, 150 mM KCl, and 15 mM Mg^{2+} when present. The association constants of Table I were used for these calculations.

Table III: Concentrations of Free Mg^{2+} and Corresponding Chemical-Shift Separations of the α- and β-Phosphorus Resonances of Intracellular ATP in Control and Ischemic-Arrested Guinea Pig Hearts[a]

	control	ischemic arrest 1	ischemic arrest 2	ischemic arrest 3
free Mg^{2+} concn (mM)	2.5 ± 0.7	2.9 ± 0.9	3.1 ± 0.7	3.0 ± 0.6
chemical shifts (ppm)				
α-P and β-P resonances	8.57 ± 0.04	8.71 ± 0.05	8.77 ± 0.04	8.79 ± 0.05
standard ATP	10.94 ± 0.01	11.30 ± 0.01	11.39 ± 0.01	11.45 ± 0.01
standard MgATP	8.43 ± 0.01	8.50 ± 0.01	8.51 ± 0.01	8.51 ± 0.01
pH	7.0	6.4	6.2	6.1

[a] The periods listed correspond to times after the initiation of ischemic arrest as follows: (1) 2–12 min, (2) 13.5–23.5 min, and (3) 25–35 min. The pH values were those determined by ^{31}P NMR measurements using the P_i line. The K^+ (Na^+) concentration used was 170 mM assuming Na^+ has the same interaction as K^+. Values represent the mean ± the standard deviation. (The errors in standard chemical shifts are estimated.)

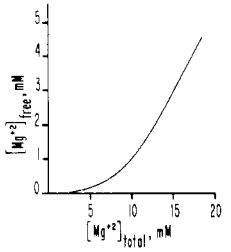

FIGURE 2: Calculation of the concentration of free Mg^{2+}. Multiequilibria calculations of free Mg^{2+} concentration based on the chemical analyses given below and the equilibria of Table I. (ATP) = 8.5 mM (21.6 ± 1.4 μmol/g dry wt); (PCr) = 11.8 mM (30.1 ± 2.2 μmol/g dry wt); (K^+ + Na^+) = 170 mM; (Cl^-) = 13 mM; (myosin) = 20 mM; (inorganic phosphate) = 1 mM (estimated from spectra); (sugar phosphate) = 1 mM (estimated from spectra). The conversion factor used for dry weight to concentration in cell water was 2.54. Values of chemical analyses represent mean ± SEM.

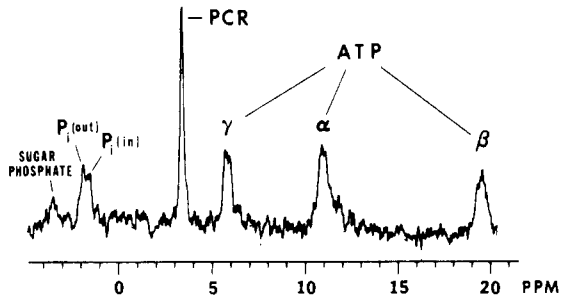

FIGURE 3: ^{31}P NMR spectrum of a control, perfused, beating guinea pig heart.

performed the multiequilibria calculations by using the equations and constants given in Table I. The results of these calculations are shown in Figure 2. If these calculations truly represent the concentration dependence, then it should agree with experimental observations based on NMR analyses which measure free cytosolic Mg^{2+} concentration and chemical analyses of total heart Mg^{2+} concentration. Thus, ^{31}P NMR measurements of perfused hearts and subsequent chemical and mathematical analyses were performed. A typical control spectrum of a perfused, beating guinea pig heart is shown in Figure 3. The effect of ischemic arrest on this spectrum has been extensively documented in a separate publication (Pieper et al., 1980). Based on the separation of the α- and β-phosphorus resonances and the calculations discussed above, we obtain a value of free cytosolic Mg^{2+} concentration of 2.5 ± 0.7 mM for control hearts (Table III).[1] Utilizing this value

[1] Hearts were perfused at 37 °C, but the stability constant used was for 25 °C. This temperature was used in order to compare our results with those of Gupta & Moore (1980). When the stability constant at 37 °C is used, the concentration of free Mg^{2+} decreases by about 0.5 mM.

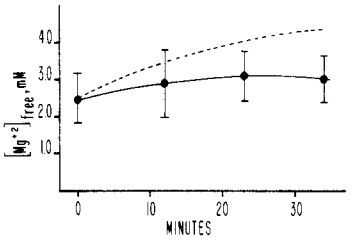

FIGURE 4: Experimentally derived and theoretically determined concentrations of free Mg^{2+} during 35 min of ischemic arrest. The points are calculated mean values from the ^{31}P NMR spectra of six guinea pig hearts. The dashed line was determined from pure theoretical calculations based upon a value of 14 mM for total cytosolic Mg^{2+} concentration of guinea pig hearts and the multiple equilibria shown in Table I.

and the theoretical plot shown in Figure 2, we see that this *measured* level of free Mg^{2+} would correspond to a *total cytosolic* Mg^{2+} concentration of about 14 mM. Our direct chemical analysis of total heart Mg^{2+} concentration by atomic absorption spectroscopy gave a value of 16.4 mM (41.7 ± 3.6 μmol/g dry weight). The ratio of *total cytosolic* Mg^{2+} concentration to *total heart* Mg^{2+} concentration shows that about 85% of the Mg^{2+} in hearts is cytosolic. This percentage appears to be in good agreement with the value determined by measurements of the Mg^{2+} present in cytosolic vs. mitochondrial fractions of heart (Polimeni & Page, 1974).

Knowing the total cytosolic concentration of Mg^{2+} at pH 7, we can now predict changes in free Mg^{2+} concentration which should occur with developing acidosis during global ischemia (Pieper et al., 1980). The dashed line in Figure 4 shows the results of these calculations based on 14 mM total cytosolic Mg^{2+}. The calculated line assumes constant total K^+ and Mg^{2+} concentration. The pH and total ATP concentrations at each time were corrected so as to correspond to the values present in the experimental hearts based on NMR measurements. When the predicted curve is compared with the experimental results, we see an increase in free Mg^{2+} concentration of a factor of 2 while the experimental results seem to be constant within the error. It is possible that this difference has to do with the assumptions in the calculated curve concerning total K^+ and Mg^{2+} concentration. If the intracellular concentrations of these ions were to decrease during the periods of ischemia, as has been suggested in some ischemic heart models (Hochrein et al., 1967; Jennings et al., 1978; Mochizuki & Neely, 1980), we might expect compensatory effects on the Mg^{2+}–ATP equilibrium leading to smaller than expected changes.

Our analysis of the free Mg^{2+} concentration in guinea pig hearts gives a concentration which would be considered too high to regulate most Mg^{2+}-dependent enzyme reactions. This result contrasts with that of Gupta & Moore (1980) for frog

Table IV: Selection of Stability Constants for MgATP and Calculated Free Mg^{2+} Concentration at Normalized Conditions[a]

method	supporting electrolyte	pH	ionic strength (M)	temp (°C)	reported app stability constant (M^{-1})	ref	normalized[a] stability constant (M^{-1})	calcd free Mg^{2+} concn (mM)[b] using normalized stability constant
competition in soln with 8-hydroxyquinoline	*N*-ethylmorpholine buffer	8.0	0.05	30	73 000	*c*	26 000	2.6 ± 0.8
	triethanolamine buffer	8.4	0.1	25	38 000	*d*	20 300	3.3 ± 1.0
	triethanolamine buffer	8.2	0.05	25	80 000	*e*	30 400	2.2 ± 0.7
resin competition	$(C_3H_7)_4NBr$	8.7	0.1	25	42 700	*f*	23 000	2.9 ± 0.9
pH titration	$(C_2H_5)_4NBr$	7.0	0.1	25	26 700	*g*	26 000	2.6 ± 0.8
^{31}P NMR	KCl	7.2	0.15	25	22 000	*h*	59 000	1.1 ± 0.3
8-hydroxyquinoline-5-sulfonate	KCl in 25 mM Hepes buffer	7.1	0.15	25	9 800	*i*	29 000	2.2 ± 0.7

[a] Normalized conditions: pH 7.0 and $I = 0.15$ M in a noninteracting supporting medium at 25 °C. For the normalization method, see O'Sullivan & Smithers (1979). [b] Free Mg^{2+} concentration calculation used eq 1 of the text neglecting the $(H^+)K_{H\text{-}ATP}$ since the pH is already corrected. [c] O'Sullivan & Perrin (1964). [d] Burton (1959). [e] Watanabe et al. (1963). [f] Phillips et al. (1966). [g] Nanninga (1961). [h] Gupta & Moore (1980). [i] From two determinations made in this laboratory as described in the text.

skeletal muscle (0.6 mM). Differences between the two tissues might explain the factor of 4 difference in free Mg^{2+} concentration. However, Nanninga (1961) obtained a calculated value for frog skeletal muscle (3.37 mM) which is much closer to ours. What is the reason for the difference between our result and the result of Gupta & Moore (1980)? Although Gupta & Moore (1980) did not consider the interactions of H^+ and K^+ with ATP, they did determine an apparent association constant for Mg^{2+} binding to ATP at pH 7.2 and 0.15 M K^+, conditions which are not greatly different from those used here in Table III. Furthermore, the chemical-shift difference reported for control spectra by Gupta & Moore (1980) for frog muscle was $\delta_{\alpha\beta} = 8.6$, which is virtually identical with that found here for control hearts (Table III). Thus, the differences in the respective "measured" values come from a difference in the stability constants used. Our calculations were based on the intrinsic values given in Table I. The Gupta–Moore value was measured by NMR (Gupta et al., 1978). It is necessary to consider how accurate this measured value is as compared with those determined by other investigators and also to attempt to directly measure the value under the same conditions as those used by Gupta et al. (1978).

We have summarized the extensive literature in this area in Table IV. In order to compare constants, it was necessary to account for the different conditions used in the measurements (i.e., pH, ionic strength, and temperature) by making accepted calculations so as to normalize these constants.

This was accomplished according to the references given in Table IV. All values, except the value of Gupta et al. (1978) quoted by Gupta & Moore (1980), are in good agreement. Calculations of free Mg^{2+} concentration for heart by using these normalized constants are in excellent agreement with our calculations, but all values differ by a factor of about 2–3 when compared with the value of Gupta et al. (1978).

When Gupta et al. (1978) measured K' with NMR, they found a value which they claimed agrees with that measured by Phillips et al. (1966). Although the two reported values certainly agree after pH correction, the agreement is misleading since Phillips et al. (1966) did not have K^+ or other interacting ions present while Gupta et al. (1978) did. As can be clearly seen in Table IV, when both constants are compared under normalized conditions, there is a considerable difference. In order to resolve the problem and eliminate any uncertainties associated with calculations, we have directly measured the MgATP stability constant under conditions similar to those of Gupta et al. (1978). The results are shown in Table IV. It is clear that the value of Gupta et al. (1978) is nearly 2-fold higher. Our normalized value agrees very well with all other determinations listed.

In conclusion, we have shown that accurate analysis of free Mg^{2+} concentration in living tissue by using the NMR method also requires a complete multiequilibria analysis of all reactions involved. Our analysis does not suggest a regulatory role for free Mg^{2+} along the lines considered by Gupta & Moore (1980) for frog skeletal muscle. However, the higher levels of free Mg^{2+} we find may be important to the metabolic processes involved in adenosine release from the heart (Rubio & Berne, 1978). Finally, we should like to point out that future improvements on our approach may be expected through computer simulation of cardiac NMR spectra based on calculations considering all theoretically possible metabolic and ionic species present. Although we would not expect dramatic differences in free Mg^{2+} concentration as compared with the value we report here, the differences could be important when studied under different experimental conditions.

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Altered Amidation of Pancreatic Polypeptide in Cultured Dog Islet Tissue†

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ABSTRACT: Three forms of immunoreactive pancreatic polypeptide (PPI) were detected in extracts of cultured dog pancreatic PP cells: PPI of (1) larger apparent molecular weight than PP, (2) similar apparent molecular weight but different isoelectric point than PP, and (3) identical apparent molecular weight and isoelectric point with PP. Dog pancreatic endocrine cells in culture were labeled biosynthetically with tritiated amino acids, and extracted proteins were fractionated by sodium dodecyl sulfate gel electrophoresis. A total of 97% of the PPI migrated like PP itself while about 3% of the PPI migrated like proteins up to of 7200 molecular weight. PPI migrating like PP was analyzed further by isoelectric focusing and was found to occur in a neutral form like PP and a more acidic form. Peptide mapping of neutral and acidic PPI forms showed that both were like PP with the exception that the C-terminal [^{3}H]tyrosine-containing peptide was a peptide with a net negative charge of 1 arising from a peptide extension of one or a few amino acids. The acidic form of PP was also shown to occur in pancreas extracts. However, neutral PPI was 90% of the total PPI in the pancreas extracts while the converse was true of culture extracts. We conclude that culturing the PP cell affects the efficiency of the process of amidation, that acidic PP could be either biosynthetic precursor or end product, and that the existence of the larger PP form(s) signals (signal) the possible production of yet other peptides by the PP cell.

Pancreatic polypeptide (PP)[1] was originally discovered as a minor component of purified insulin (Lin & Chance, 1972; Kimmel et al., 1975). It appears to be a hormone in that it is found in the pancreas in high concentrations (Gersell et al., 1979) and derives from a unique pancreatic endocrine cell type (Greider et al., 1978). Furthermore, it has an amidated C-terminal like many biologically active peptides and exhibits plasma concentration variations suggestive of a role in the postprandial state (Floyd et al., 1977). However, PP falls short of qualification as a hormone since no physiological role for it has been identified. The principal potential target tissue appears to be the pancreas itself where PP can inhibit sec-

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[1] Abbreviations used: PP, pancreatic polypeptide; pPP, porcine pancreatic polypeptide; PPI, pancreatic polypeptide immunoactivity measured by radioimmunoassay and expressed in porcine pancreatic polypeptide equivalents; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane. A major form of pancreatic polypeptide immunoactivity in culture extracts which migrated to near pH 5 in isoelectric focusing is referred to as acidic PPI to distinguish it from immunoactivity which migrated to near pH 7 as did pPP.

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