Brief Notes and Comments

Potentiation of Insulin Action by Calcium and Magnesium

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Recent experiments have shown that commercial insulin produces an increase in short circuit current across the isolated short-circuited frog skin.[1] This in vitro phenomenon was observed only in the presence of Ca^{++} and Mg^{++} which suggested that these ions were necessary for the action of insulin in such an experimental system. The present study utilizing intact mice was designed to determine the effect of added Ca^{++} and Mg^{++} in an in vivo system.

METHODS

Insulin activity was determined by the technic of the classical mouse convulsion method of bioassay as described by Treven and Boock[2] and Hemmingsen and Krogh.[3]

Swiss albino mice weighing from 18 to 23 gm. were used in the present experiments. The mice were fasted for more than three hours previous to the insulin injection.

Three experimental groups were designed as follows:

1) *Controls.* Glucagon-free insulin with a concentration of 40 I.U. per ml. was dissolved in saline solution and at *p*H 3.5 to obtain concentrations of 0.008; 0.01; 0.02 and 0.03 I.U. in each 0.25 ml. of the solution, in which amounts it was injected in the dorsum of each mouse. Immediately after the injection of insulin the mice were put into glass jars in an environmental temperature of 34° C. and observed during a two-hour period for convulsions.

2) *Pretreatment with calcium gluconate.* In this group, all mice were treated with 0.15 ml. of a 10 per cent solution injected intraperitoneally one hour prior to the injection of insulin in the same dosages as the control mice received.

3) *Pretreatment with magnesium sulfate.* This experimental group was the same as group 2 except that pretreatment with 0.15 ml. of 1.5 per cent magnesium sulfate solution intraperitoneally was carried out two to three hours prior to insulin.

Prior to the injection of insulin, the mice did not display any observable pharmacological effect to the injection of calcium gluconate and magnesium sulfate in the doses and volumes used in these studies, peritoneally. However, it was found that magnesium sulfate needed a longer equilibrium period to produce the results observed.

The results of these experiments were analyzed statistically according to the procedure recommended by the League of Nations[4] following the procedure recommended by Trevan and Boock[2] and Hemmingsen and Krogh.[3] When the formula recommended by these authors was applied for the action of the concentrations 0.01 and 0.02 I.U. it was found that a brand of insulin with 40 I.U. per ml. had increased to the activity of 60 I.U. per ml.

The formula for this type of statistics is as follows:

$$\mathbf{M} = \frac{Cm - Cp}{C_2 - C_1} \log 2 + \log M_1,$$

where:

- **Cm** = Number of mice in both groups which received insulin U. 40 plus Ca^{++} or $Mg.^{++}$
- **Cp** = Number of mice with convulsions which received insulin alone, both groups included.
- $\mathbf{C_1}$ = Number of mice with convulsions which received 0.01 I.U. insulin either with or without calcium or magnesium.
- $\mathbf{C_2}$ = Number of mice with convulsions which received 0.02 I.U. insulin either with or without calcium or magnesium.
- $\mathbf{M_1}$ = activity of the insulin used (40 I.U.).
- **M** = crude potency of the problem sample (insulin plus Ca or Mg).

The antilogarithm of M shows in international units, the potency of the sample problem. In this case the mixture of insulin plus calcium increased the action of the

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TABLE 1

	Amount of insulin I.U.	Number of mice	Number of convulsions observed	Per cent convulsions	*P*
Controls	0.005	20	0		
	0.008	20	0		
	0.01	50	3	6	
	0.02	80	33	41	
	0.03	20	20	100	
Pretreatment with 0.15 ml. of 10 per cent Ca Gluconate					
	0.005	20	0		
	0.008	20	9	45	
	0.01	30	11	37	<0.001
	0.02	50	32	64	<0.05
	0.03	20	20	100	
Pretreatment with 0.15 ml. of 1.5 per cent $MgSO_4$					
	0.005	20	0		
	0.008	20	0		
	0.01	50	17	34	<0.01
	0.02	49	31	63	<0.05
	0.03	20	20		

glucagon-free insulin used from 40 to 60 I.U. The same procedure was utilized for the calculation of the potentiation with $Mg,^{++}$ the same results having been found.

For this reason it was decided to continue experiments to find the range of dosages of insulin in which the potentiation of insulin by these di-valent ions appeared. Statistics follow the chi-square procedure.

RESULTS

The results of these experiments are shown in the accompanying table. The addition of insulin clearly potentiated the convulsant action of insulin to a statistically significant degree when 0.01 I.U. of glucagon-free insulin was used and of borderline significance when 0.02 I.U. was used. Moreover, this effect with calcium could even be observed when only 0.008 I.U. of insulin was used.

DISCUSSION

It has been shown that the addition of Ca^{++} and Mg^{++} ions produced a true potentiation of the *convulsive* activity of insulin in mice. The difference between the range of activity of the two ions seems to show that we are in the presence of a true pharmacological phenomenon. Although it seems likely that this potentiation is due to an increase in hypoglycemia, the exact mechanism is not clear on the basis of these preliminary studies which only intend to register the phenomenon. Other mechanisms such as a lowering of the CNS convulsive threshold or of transmembrane potentials, as well as dosage-dependent relationships are currently being investigated.

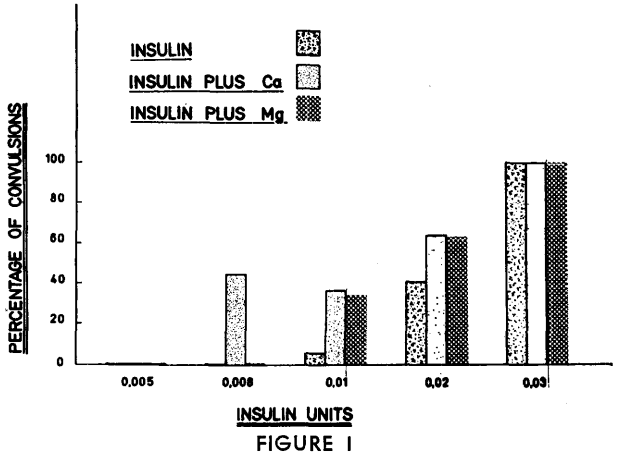

FIGURE 1

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