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EFFECT OF INSULIN ON THE INH* TRANSPORT ACROSS THE CELL MEMBRANES IN THE PERFUSED LIVER AND ISOLATED HEMIDIAPHRAGM OF RATS†

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Abstract—The effect of insulin on INH transport across the cell membranes in the perfused liver and isolated hemidiaphragm or rats was studied. Insulin does not affect the rate of destruction of INH by liver tissue. Insulin increased the transport of INH from perfusate into the tissue as well as the elimination from tissue into the bile. In experiments with isolated hemidiaphragm insulin increased the transport of INH-C^{14} into the intracellular space.

IT IS generally accepted that insulin modifies cell membrane function in such a way as to increase the velocity of penetration of certain sugars and amino acids.[1–9]

In previous investigations we have found that administration of insulin (together with sufficient sugar to prevent hypoglycemia) with certain drugs increased both the tissue contents and the pharmacological effects of these drugs.[10–17] We also found[11] that insulin given intracardially with INH increased its level in brain and liver tissue, despite the fact that the blood-brain barrier and the cell membrane of liver are insensitive to the hormon as far as glucose penetration is concerned.[18–22]

In order to establish that the insulin effects observed *in vivo* were due to a direct action on the tissues examined, and to determine to what extent changes in the rate of drug destruction were involved, it was deemed valuable to examine the effect of insulin on the transport and destruction of INH in the perfused rat liver, and also the effect of insulin on the transport of INH-C^{14} into the intracellular space of hemidiaphragm.

MATERIALS AND METHODS

Perfusion of liver. The experiments were carried out on fasting (18 hr) male rats.

Liver donors were Wistar rats, weighing 250–320 g, and blood donors, Sprague–Dawley retired breeders (400–600 g). A slight modification[23] of Miller's[24] technique was used to perfuse the liver. The perfusion mixture comprised 80–100 ml of blood freshly withdrawn, one-third its volume of Ringer's solution and 45 mg heparin. The circulating blood (mixture) was continuously oxygenated with 95% O_2 + 5% CO_2. INH (60 μg/ml) or INH with Insulin (Lilly, lot No. PJ-5589—0·01 U/ml) were added to the perfusate. INH was determined by the Deeb and Vitagliano method.[25]

* Isonicotinic Acid Hydrazide.

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Isolated hemidiaphragm. Male rats of the Wistar strain, weighing 90–140 g, fasted 18 hr, were used in all experiments. The rat hemidiaphragm was prepared according to Gemmil.[26] One hemi-diaphragm was used as a control, while the other was incubated with insulin and the insulin effect noted in each pair of incubations. The hemidiaphragms were placed in 4 ml of Krebs–Ringer bicarbonate buffer which was continusouly oxygenated with 95% O_2 + 5% CO_2. The incubations were carried out at 37° in a Dubnoff metabolic shaker. After 15 min of adaptation the following reagents were added to the buffer:

Control vessel: INH-C^{14} (Nuclear Chicago) 0·05 μc/ml + Sorbitol-H^3 (Nuclear Chicago) 1·0 μc/ml.

Insulin vessel: INH-C^{14} + Sorbitol-H^3 + Insulin (Lilly, 0·01 U/ml).

Sorbitol-H^3 was used for determination of the extracellular space.[27]

The intracellular concentration of INH-C^{14} was determined from the following formula.[28]

$$Ci = \frac{Ct - Cm \times (Ss/Sm)}{1 - (Ss/Sm)}$$

Ci = concentration of INH-C^{14} in μc/ml of intracellular water
Ct = concentration of INH-C^{14} in μc/ml of tissue water
Cm = concentration of INH-C^{14} in μc/ml of incubation medium
Ss = concentration of Sorbitol-H^3 in μc/ml of tissue water
Sm = concentration of Sorbitol-H^3 in μc/ml of incubation medium

After incubation, the tissue was blotted, weighed, and then digested in 0·5 ml Solubilizer (NCS reagent model 19060, Nuclear Chicago) for 2 hr at 60°. The tube contents were then transferred to a scintillation counting vial, to which were added 4 ml methanol and 10 ml toluen containing 4 g 2,5-diphenyloxazole (PPO) and 50 mg 1,4-di-2-(5-Phenyloxazolyl) benzene (POPO) per l. The buffer (0·1 ml) was assayed for radioactivity after addition of 4 ml Methanol with 15 ml Toluen with P.P.O. and P.O.P.O.P. The samples were assayed for C^{14} and H^3 in a Packard Liquid scintillation spectrophotometer*. Quenching in each sample was determined with an external standard.

RESULTS AND DISCUSSION

Liver perfusion experiments. When a single dose of insulin was added to the initial perfusate together with INH, the content of INH in the liver tissue after 15 min perfusion was higher (18·3 ± 1·68 μg/g) than in controls without insulin (11·5 ± 1·05 μg/g; $P < 0{\cdot}01$) (Fig. 1).

The difference became smaller at 30 min, and disappeared by 45 min. The rate of destruction of INH by liver tissue was very fast.

During 45 min of perfusion about 35 per cent (i.e. about 2 mg) of the administered dose of INH was destroyed. This process was not affected by insulin (Fig. 2).

Because the blood level of INH at 15 min was at least three times higher than that of the liver, a large portion of the drug found in the liver was extracellular, possibly obscuring an insulin effect on intracellular penetration. To correct this in several experiments the liver after 15 min of perfusion was washed with 20 ml of the Ringer solution, immediately after the usual 15-min samples were taken.

* Kindly made available by Dr. Sara Schiller, Department of Pathology, New York Medical College.

It was found (Table 1) that about two-thirds of the INH was washed out of the liver in all experiments. In the experiments with insulin a twice higher concentration of INH in the liver tissue was still observed.

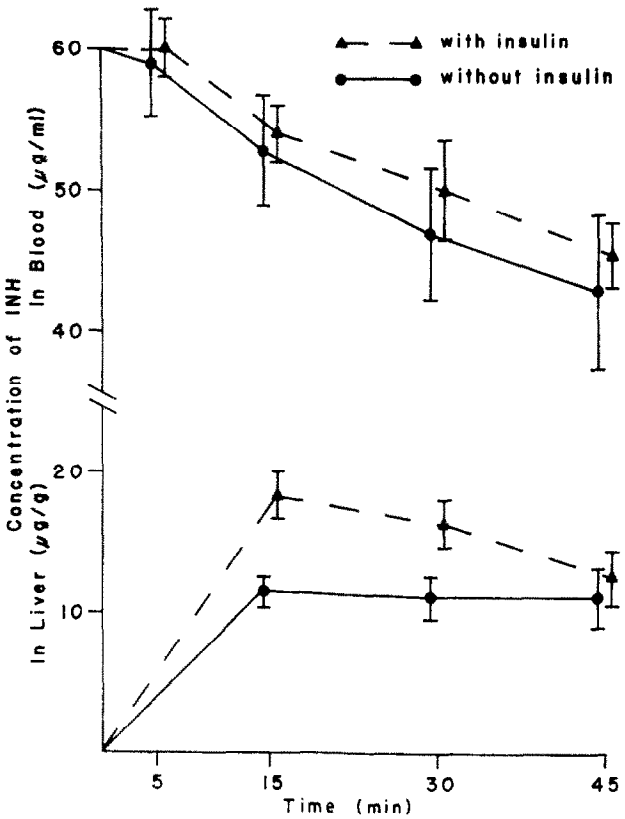

FIG. 1. The influence of insulin on INH transport into the perfused liver. Each point is the mean value of 6 experiments.

TABLE 1. EFFECT OF INSULIN ON THE INH TRANSPORT INTO THE PERFUSED LIVER

No. of exp.	The concentration of INH in μg/g of the liver tissue in exp. with insulin		The concentration of INH in μg/g of the liver tissue in exp. without insulin	
	Unwashed	Washed	Unwashed	Washed
1	18·0	6·0	13·0	3·0
2	18·0	9·0	12·0	4·0
3	18·0	6·0	10·0	3·0
Average	18·0	7·0	11·0	3·3

Although the results suggest that insulin increased the velocity of the INH transport from the perfusate into the liver tissue, it was necessary to explain why the effect of insulin was observed only at the beginning of the perfusion. The effect may have been transient because of rapid destruction of the hormone in the liver. Another possibility was that the elimination of INH was higher in experiments with insulin.

In the further experiments insulin was given continuously (0·01 U/ml 90 min) and the content of INH measured in bile collected during perfusion.

The effect of insulin, continuously administered on the concentration of INH in the liver tissue was essentially the same as in the previous experiments (Fig. 3).

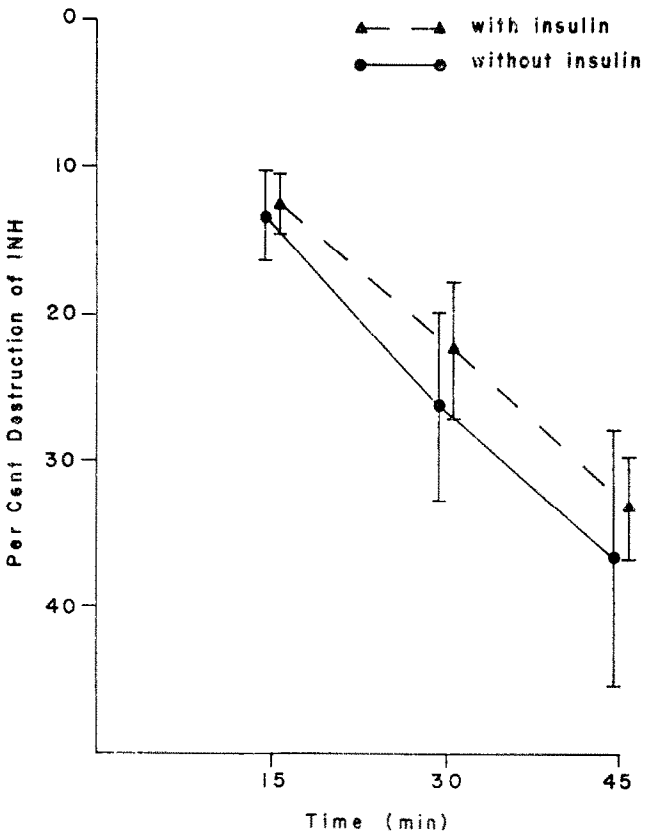

FIG. 2. The influence of insulin on INH destruction by the liver tissue. Each point is the mean value of 6 experiments.

Insulin almost doubled (from — 37·8 ± 4·34 μg/ml in control to 55·8 ± 3·48 μg/ml, $P < 0·01$) the concentration of INH in the bile without any effect on the volume of the collected bile (mean: 0·80 ml of bile in control experiments and 0·82 ml in experiments with insulin). The difference in the content of INH in the bile was of the same order as the difference in the total liver content at 15 min perfusion.

We can say that in experiments with insulin not only transport of INH into the liver tissue is increased but also the drugs elimination into the bile.

The rate of the INH destruction was also unchanged by insulin (Fig. 4).

However it was still obscure why the "transport" effect of insulin was observed only at the beginning of perfusion. Considerations was given to the possibility that the insulin effect occurs only while INH is maintained at high concentration in the blood. Therefore in the further experiments both insulin and INH were given continuously. INH was added at 0 time—60 μg/ml and it was administered during

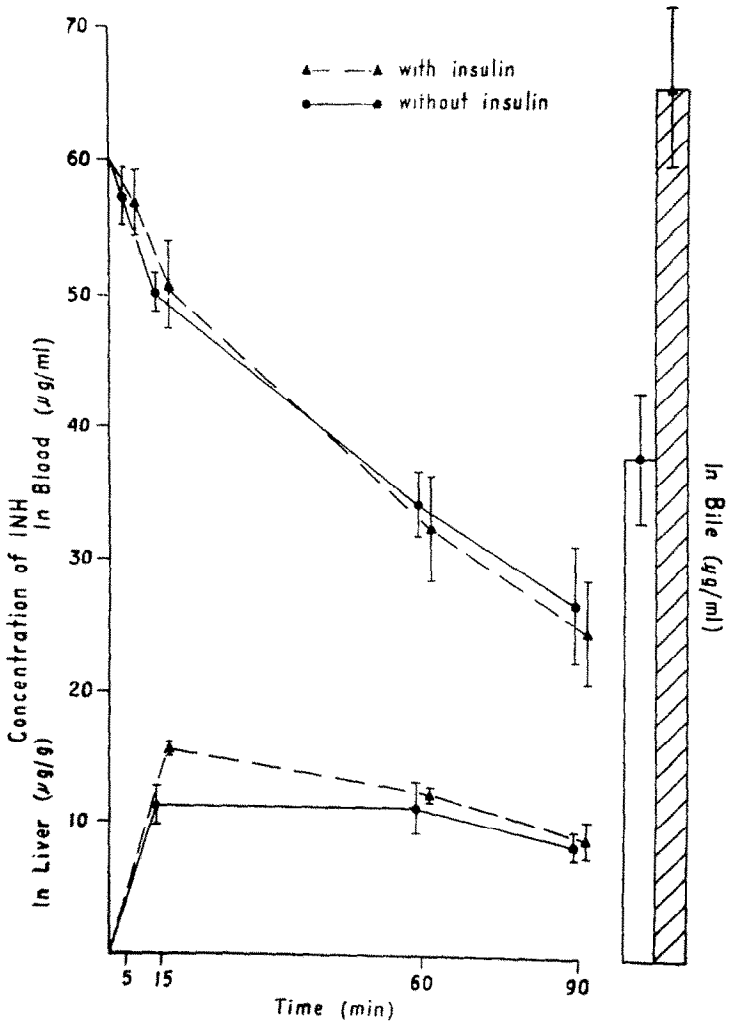

FIG. 3. The influence of insulin on INH transport into the perfused liver Insulin was given continuously. Each point is the mean value of 6 experiments.

perfusion at the rate of 3·0 mg/90 min. which corresponded to the rate of destruction noted previously. This maintained a uniform level in the perfusate for 90 min.

Under these conditions the insulin effect on liver concentration of INH was sustained throughout the experiment (Fig. 5).

It seems that the transport effect of insulin can be observed only when the level of INH in the perfusate is high.

The concentration of INH in bile in the experiments with insulin was twice higher (86·6 $\pm$ 9·3 μg/ml) than in control (48·0 $\pm$ 5·5 μg/ml, $P < 0{\cdot}01$), the volume of the bile being unaffected by insulin. With insulin the concentration of INH in the bile was almost 2 times higher than in the perfusate, suggesting that insulin increased the active elimination of the drug.

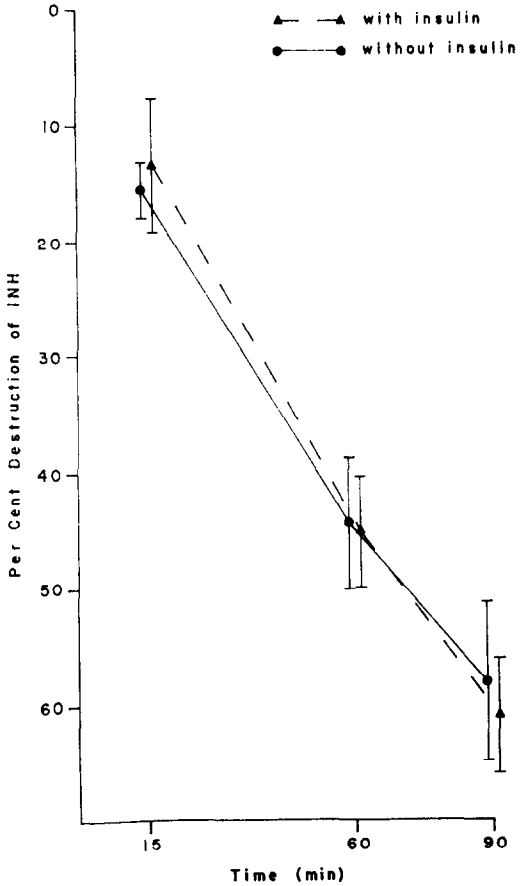

FIG. 4. The influence of insulin given continuously on INH destruction by the liver. Each point is the mean value of 6 experiments.

TABLE 2. EFFECT OF INSULIN ON THE TRANSPORT OF INH-C^{14} INTO THE INTRACELLULAR SPACE OF THE ISOLATED HEMIDIAPHRAGM

No. exp.	Time (min)	Insulin			No insulin		
		Ss/Sm	*Cm*	*Ci*	*Ss/Sm*	*Cm*	*Ci*
1		0·42	0·0277	0·0098	0·43	0·0282	0·0086
2		0·28	0·0251	0·0051	0·31	0·0238	0·0042
3	30	0·35	0·0244	0·0087	0·30	0·0250	0·0078
4		0·41	0·0250	0·0054	0·38	0·0273	0·0050
5		0·40	0·0247	0·0091	0·40	0·0256	0·0082
6		0·40	0·0262	0·0088	0·35	0·0300	0·0082
7		0·43	0·0270	0·0104	0·45	0·0276	0·0087
8		0·43	0·0251	0·0102	0·50	0·0302	0·0072
9	60	0·39	0·0244	0·0061	0·36	0·0264	0·0038
10		0·40	0·0250	0·0056	0·41	0·0251	0·0043
11		0·48	0·0247	0·0107	0·42	0·0262	0·0090
12		0·44	0·0262	0·0098	0·49	0·0265	0·0071
13		0·44	0·0239	0·0017	0·47	0·0264	0·0011
14		0·42	0·0280	0·0024	0·39	0·0284	0·0005
15	90	0·43	0·0262	0·0077	0·44	0·0264	0·0075
16		0·54	0·0250	0·0101	0·52	0·0271	0·0089
17		0·52	0·0250	0·0089	0·50	0·0241	0·0083
18		0·46	0·0256	0·0060	0·48	0·0259	0·0040

Hemidiaphragm experiments

In the experiments with isolated hemidiaphragm it was found that insulin increased the intracellular content of INH-C^{14} in every experiment (Table 2), whereas it had no effect on the volume of the extracellular space, as indicated by the penetration of sorbitol.

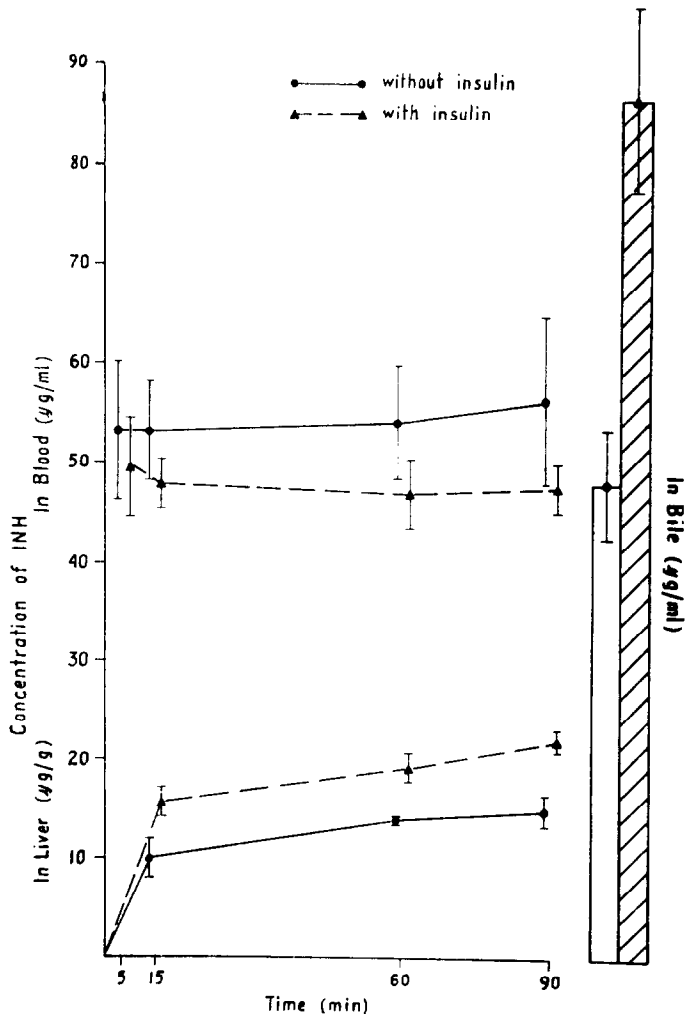

FIG. 5. The influence of insulin on INH transport into the perfused liver. Insulin and INH were given continuously. Each point is the mean value of 6 experiments.

It was found in previous investigations[10–17] that insulin given with many drugs increased the potency of their action and their content in tissues. The above results show that, enhanced tissue level of the drugs after insulin administration does not depend on a change in drug metabolism but on an increase in the rate of drug transport into the tissue.

The problem of sensitivity of the liver cell membrane for insulin has been considered for a long time. Although insulin has been shown to have reproducible effects on net glucose exchange by the perfused liver.[23] The data are independent of any effect of insulin on glucose penetration, and in fact glucose penetration itself seems not to be affected by insulin.[22] Our results presented above show that insulin increased the transport of INH through the cell membrane of the liver tissue. The insulin can increase the transport of drug not only across different cell membranes (e.g. blood vessels-tissue, blood-brein barrier) but also through the cell membrane into the intracellular space of the hemidiaphragm.

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