INJURY-STIMULATED UPTAKE OF α-AMINOISOBUTYRIC ACID BY RAT LIVER

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SUMMARY

Injury stimulated an early increased uptake of α -aminoisobutyric acid (AIB) in rat liver. Adrenalectomy yielded a delayed stimulation reaching maximum levels at 48 hours post operation. Chronic adrenalectomized rats were hyper-sensitive to stimulus of reinjury and injections of epinephrine or hydrocortisone.

Injury stimulates the hepatic synthesis of certain plasma proteins, a process which is dependent upon an elevated formation of RNA and an increased aggregation of polysomes (1-3). This chain of events may be triggered by specific stress factors and may be dependent on a permissive effect of glucocorticoids (4). If these events in the liver are set in motion by factors transported by the blood stream, it would be of special value to ascertain if the entire process is preceded by a significant change in the permeability of the liver cells. That this supposition is likely was shown in determinations of the distribution of hepatic amino acids following partial hepatectomy (5).

In the following report we describe an early, elevated uptake by rat liver of the model amino acid, α -aminoisobutyric acid (AIB), following sham operation, adrenalectomy, and hormone replacement therapy. AIB is not metabolized (6,7) so that its accumulation in the cell can be considered an index of changes in permeability. Furthermore, its cellular uptake depends on an active transport mechanism (8).

MATERIALS AND METHODS

Male, Sprague-Dawley rats, weighing 160-200 gms, were fed *ad libitum*. Injury was performed under ether anesthesia by sham adrenalectomy except where otherwise indicated. Rats were adrenalectomized using a dorsal approach. These animals were then given 0.9% saline solution for drinking water.

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Rats were injected intravenously via a tail vein with 1 μ Ci/100 gm body weight of ^{14}C-AIB (53.8 mCi/mM, Amersham/Searle). After 60 minutes, the rats were decapitated, the livers were perfused with saline, removed, chilled, and minced in cold Tris buffer (0.44 M sucrose, 0.05 M Tris-HCl, 0.025 M KCl, and 0.005 M $MgCl_2$), pH 7.5. An aliquot, 0.1 ml, was combined with 5 ml of scintillation mixture (9) and counted in a liquid scintillation counter (80% efficiency). The data are expressed in terms of the percentage of radioactivity recovered; cpm in the liver/cpm injected X 100.

Epinephrine (1:1000 Adrenalin HCl, Parke Davis) was injected subcutaneously. Hydrocortisone-sodium succinate (Solu-Cortef, Upjohn) was injected intraperitoneally as an aqueous solution, 1 mg/ml, in doses of 1 mg/100 gm body weight.

RESULTS AND DISCUSSION

Injury elicits a considerable enhancement in the uptake of AIB by the rat liver. A preliminary study, using pulses of 15, 30, 60, and 90 minutes showed that the uptake in normal rats was gradual and linear (Table 1). The uptake in 4 hour injured rat livers was linear but stimulated between 2 and 4 fold. Subsequently a pulse of 60 minutes was used for all studies. Figure 1 shows the change in the stimulated uptake with time after sham operation. It is clear that the maximum effect occurred from 4 to 8 hours post injury.

TABLE 1. RECOVERY OF α-AMINOISOBUTRYIC ACID-1-^{14}C AND ^{14}C-INULIN IN LIVERS FROM NORMAL AND 4 HOUR-INJURED RATS.

Treatment		Percentage Recovery at Various Pulses (minutes)*			
		15	30	60	90
^{14}C-AIB					
	Normal	3.5	4.2	4.7	5.5
	4 hour Injury	7.7	11.3	15.6	21.9
^{14}C-Inulin					
	Normal	1.8	1.8	1.7	—
	4 hour Injury	2.0	1.8	1.4	—

*Data represent averages of 2-4 rats at each point.

To prove that this response to injury actually involved cellular uptake and not an increase in extracellular space, 2 experiments were performed. In the first, the perfused livers of 2 normal and 2 injured rats were minced and then carefully forced through 100 mesh nylon cloth to yield suspensions of cells in cold 0.2 M sucrose-Tris buffer. The suspensions were centrifuged at low speed

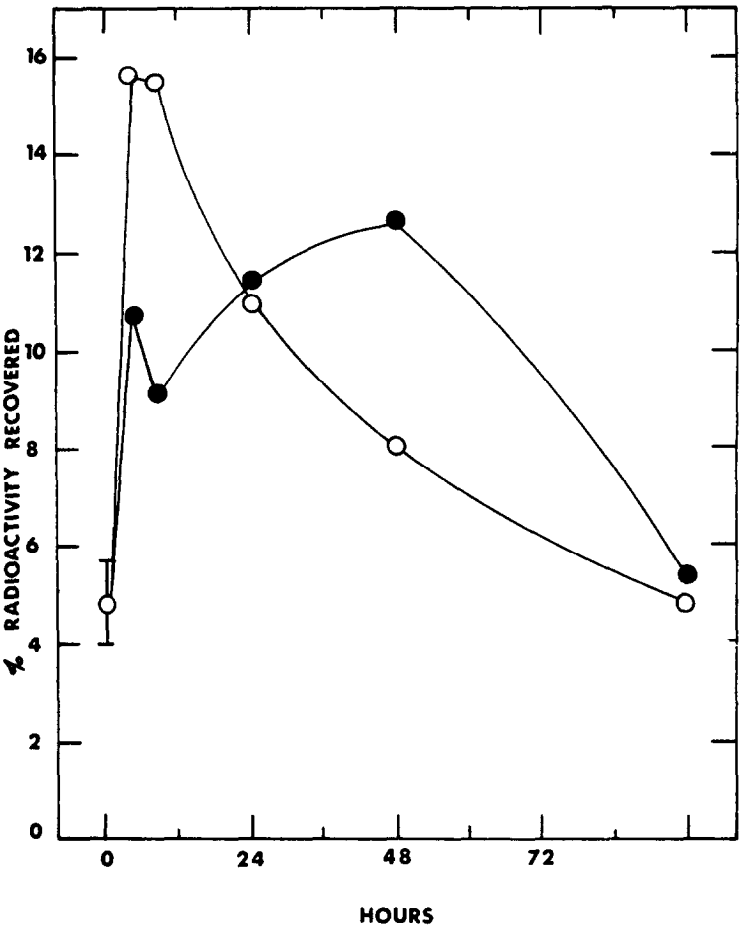

Figure 1. Changes in ^{14}C-AIB uptake in the livers of injured (O) and adrenalectomized (●) rats. Ordinate is the percentage of ^{14}C-AIB injected which was recovered in the total liver. Abscissa is the time after operation. AIB was injected 60 minutes before sacrifice.

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and the cells were washed in fresh, cold sucrose medium. After centrifugation, the sedimented cells were ruptured in 1 ml of 0.1N NaOH. Aliquots of 0.5 ml were combined with 5 ml of scintillation fluid and counted; the remainder of the samples was used for determinations of total protein (10). Cells from 4-hour injured rats contained 122% more radioactivity per mg of protein than did the normals (average of 315 compared with 142 cpm/mg of protein). Therefore, the stimulated uptake of AIB was associated with the cells. In a second experiment, normal and injured rats (4 hours) were given ^{14}C-inulin. The livers in this experiment were not perfused to remove blood but were otherwise treated as for the AIB studies. Table 1 shows that injury had no effect on the amount of radioactivity in the tissues. These data show that the response to injury was not the consequence of changes in extracellular space because inulin does not enter the cells.

Since the adrenals are thought to be important in mediating the effects of stress in general, AIB incorporation into livers was also investigated using adrenalectomized rats. Adrenalectomy completely modified the response profile, Figure 1. Although the effect of injury was not abolished, the profile suggests a delayed response with a maximum effect at 48 hours. Therefore the response does not seem to have an absolute requirement for the adrenal glands. Confirmation was gained from studies of reinjured, chronically (4 days) adrenalectomized rats. The data of Table 2 show that reinjury (4 hours) stimulated an immediate and even higher response than 4 hour injury alone. It seems likely that once the response mechanism had been set in motion after adrenalectomy, it was more sensitive to a second stimulus. The adrenal might serve as an initiator of increased permeability but alternate pathways may also exist. Replacement therapy with both epinephrine and hydrocortisone elicited a response at 4, 48, and 96 hours post adrenalectomy, Table 2. These results are in accord with others (12-14). It is of interest to note that the rats became increasingly responsive to these hormones with time after operation. This confirms the observation that the 96 hour adrenalectomized rats were hypersensitive to stimuli. Although the similarity of effects

TABLE 2. FACTORS INFLUENCING UPTAKE OF AIB BY LIVERS OF INJURED AND ADRENALECTOMIZED RATS.

Injuries were sham operations to adrenalectomy except for the reinjury of experiment IV which was a midline laparotomy. From 3 to 5 rats were used in each determination.

Experiment	Treatment	Average Percent Recovery
I	Normal	4.9
	Sham - 4 Hr.	15.6
	Adx. - 4 Hr.	7.6
	+ Ep.[1]	13.0
	+ HC[2]	20.9
II	Sham - 48 Hr.	8.0
	Adx. - 48 Hr.	13.0
	+ Ep.[1]	16.0
	+ HC[2]	20.2
III	Sham - 96 Hr.	4.9
	Adx. - 96 Hr.	5.4
	+ Ep.[1]	22.1
	+ HC[2]	27.8
IV	Normal	5.0
	Sham - 96 Hr.	4.9
	Adx. - 96 Hr.	5.4
	+ Reinjury - 4 Hr.	18.0
	Laparotomy - 4 Hr.	12.0

[1] 0.1 mg epinephrine/100 gm injected subcutaneously 2 hours before sacrifice.
[2] 1 mg hydrocortisone/100 gm injected intraperitoneally 4 hours before sacrifice.

observed here suggest that injury and the specific hormones tested act on a common target, it is premature to speculate further as to the nature of this response mechanism.

It is our view that the response to injury leading to an elevated synthesis of certain plasma proteins involves an early increased permeability of liver cells to amino acids. Increased pools of amino acids may be an important factor not only in supplying the necessary precursors for stimulated protein synthesis but also may be important in stabilizing polysomes (15) and thereby controlling the duration of the response.

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