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31 May 1963

## Radioprotection by Pressor Amidines

Abstract. *In the mouse, radioprotection is not always associated with the effect of hypertensive amidines and related amines. The protection resulting from this group of agents follows the pharmacological reduction of intercellular oxygen tension.*

After the observation that simple *S*-alkyl isothiuronium salts decrease radiosensitivity, Ashwood-Smith (*1*) tested some of its homologs in an attempt to relate structure to radioprotective action and to discover more promising agents. He found that activity diminishes rapidly as the *S*-alkyl substituent is lengthened beyond three carbon atoms. It is interesting that Fastier (*2*), in his excellent review of the structure-activity relationships of amidines, describes a loss of pressor action for *S*-alkyl isothiuroniums with alkyl substituent longer than three carbon atoms. The possible correlation of chemical structure, pressor activity, and radioprotection by these amidine derivatives led to a study of the effects of pressor amidines and pharmacologically related amines on the radiosensitivity of mice.

Table 1. Thirty-day survival data of mice receiving single doses of related pressor amines and amidines before irradiation to lethal doses of $Co^{60}$ (1000 r).

| Intraperitoneal administration | | Animals (No.) | Survival (%) |
|---|---|---|---|
| Dose (mg/kg) | Time (min) | | |
| | *Controls* | | |
| | | 290 | 0 |
| | *2-Methylpseudo urea* | | |
| 500 | 15 | 20 | 50 |
| | *Methyl guanidine* | | |
| 150 | 5 | 20 | 5 |
| | *2-Amino pyridine* | | |
| 25 | 15 | 30 | 0 |
| | *4-Amino pyridine* | | |
| 3 | 15 | 15 | 0 |
| | n-*Pentylamine* | | |
| 50 | 15 | 10 | 0 |
| | n-*Hexylamine* | | |
| 40 | 15 | 10 | 0 |
| | S-*ethyl isothiuronium* | | |
| 150 | 30 | 40 | 98 |
| 150 | 15 | 80 | 90 |
| 75 | 15 | 30 | 90 |
| 20 | 15 | 20 | 60 |
| | *Papaverine · HCl** | | |
| 325 | 30 | 10 | 10 |
| *Papaverine · HCl,* plus* S-*ethyl isothiuronium* | | | |
| 325 | 30 | | |
| 150 | 15 | 26 | 40 |
| | *Hypoxia*† | | |
| | | 42 | 5 |
| *Hypoxia, plus* S-*ethyl isothiuronium*† | | | |
| 150 | 15 | 20 | 5 |

* Subcutaneous administration. † Irradiated with 2200 r $Co^{60}$.

Young female mice (Bagg Swiss), weighing 20 to 25 g, were used. Ten control mice were irradiated simultaneously with each treated group and thereafter both groups were housed jointly. The radiation was done in a specially designed cobalt-60 irradiator which contained about 1200 curies of cobalt-60, half above and half below the radiation chamber. The mice were exposed in a plexiglass box which rotated through a flat radiation field of about 100 r/min. In the experiment with hypoxia, two treated and two control mice were irradiated simultaneously in a cobalt-60 Gammacell-220 (*3*) at about 1800 r/min. The irradiation chamber was gassed before and during exposure with a mixture of 5 percent oxygen and 95 percent nitrogen.

Each of the chemicals tested is known to increase blood pressure (*2*), but only two of these offered significant protection against lethal radiation. The survival data in Table 1 indicate that radioprotection by amidines is not directly associated with their pressor activity. In an attempt to explain this disparity, additional investigations were conducted with *S*-ethyl isothiuronium as a test compound.

The results in Table 1 show that *S*-ethyl isothiuronium is radioprotective when used over a wide dose range and for a considerable period of time. Also, papaverine, a known pharmacological antagonist (*2*) significantly reduced the protective effect of a massive dose of *S*-ethyl isothiuronium. Other agents—reserpine, atropine, phenergan, and dibenzyline—had no influence on *S*-ethyl isothiuronium action. The favorable therapeutic ratio and the response to a specific antagonist are parallel to actions established for serotonin (*4*), which is thought to decrease radiosensitivity through oxygen-dependent pathways. A similar mechanism may explain the action of *S*-ethyl isothiuronium since our data show that it fails to increase the radioprotection afforded mice by the optimal reduction of intercellular oxygen.

The experimental results suggest that pressor amidines offer radioprotective activity through a pharmacological mechanism which leads to a lowered oxygen tension of radiosensitive tissues.

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15 April 1963

## Glycogen Deposition in the Liver Induced by Cortisone: Dependence on Enzyme Synthesis

Abstract. *The deposition of liver glycogen in starved rats given a single dose of cortisone is inhibited by puromycin and actinomycin. The former agent interferes with induced enzyme formation in general, and the latter with the cortisone-induced rise in liver enzyme levels. The results suggest that the regulatory effect of cortisone on carbohydrate metabolism may be brought about by its action on the cellular concentration of certain enzyme proteins.*

Adrenocortical hormones, which influence the rate of certain metabolic processes in vivo, do not appear to act as simple inhibitors or activators of enzymic reactions in vitro. Therefore, Knox, Auerbach, and Lin (*1*) suggested that hormone action may be brought about by changes in the actual concentration of the protein moiety of specific enzyme systems. The dependence on enzyme synthesis of the acute stimulation of glycogen deposition by cortisone in the liver of starved rats has now been tested.

Recent data suggest that the rise of enzyme activity induced by cortisone reflects an increase in the rate of *de novo* enzyme synthesis. The accumulation of liver tyrosine transaminase (*2*), glutamic-alanine transaminase (*3*), and tryptophan pyrrolase (*4*) has been measured immunochemically. Correspondingly, the administration of an inhibitor

of protein synthesis, puromycin, prevents the induction by cortisone of such enzymes (*5*), as well as that of others concerned with gluconeogenesis (*6*). Puromycin also lowers the level of liver glycogen in normal mice (*7*).

Actinomycin, an inhibitor of RNA synthesis (*8*), also interferes with the rise of liver enzymes induced by cortisone. This suggests that the stimulation of the synthesis of certain RNA species may be one of the primary actions of the hormone (*5*). The effect of actinomycin is more specific than that of puromycin; it interferes with the increase in liver tyrosine transaminase and tryptophan pyrrolase which is brought about by cortisone, but it does not interfere with the rise in the amount of trytophan pyrrolase induced by the substrate.

We have now asked the question whether the prevention of the cortisone-induced rise in liver enzyme levels will inhibit the stimulation of glycogen deposition by the hormone. Therefore, cortisone was administered to starved rats, alone or in conjunction with the aforementioned inhibitors, and the amount of liver glycogen was estimated 6 hours later. Puromycin prevents the cortisone-induced rise in glycogen levels and actinomycin inhibits it by approximately 70 percent (Table 1). The differences observed are highly significant statistically. None of the treatments influenced the total liver or body weight significantly. To illustrate the effect of the same treatments on an individual enzyme, the tyrosine transaminase activity of some of the livers is also included in Table 1. Puromycin and actinomycin largely prevented the cortisone-induced elevation of this enzyme (*5*).

It is not known which of the enzymes controlled by cortisone regulate the rate of glycogen deposition in the liver. Some of them have a direct effect on gluconeogenesis (*9*) or on the regulation of glucose concentration (*10*). The elevation of the level of transaminases may be responsible for enhanced gluconeogenesis (*11*, *12*). The rate of increase of the activity of some of these enzymes is significantly lower than the rate of glycogen deposition, but it is possible that the small initial rise which does coincide with rapid glycogen deposition is important. It is possible that the amounts of other enzymes whose response to cortisone treatment has not yet been studied will prove to be more important in the regulation of glycogen deposition. We did not attempt to evaluate the role of individual enzymes but demonstrated that substances which generally prevent the cortisone-induced rise in liver enzyme levels also inhibit the cortisone-induced glycogen deposition. Such inhibition would not be anticipated if cortisone controlled glycogen deposition by influencing the speed of catalytic processes without alteration of enzyme amounts. Thus, these results are compatible with the suggestion (*1*) that hormonal regulation of metabolism in vivo may be brought about by changes in enzyme concentrations. Such a dependence on protein synthesis would explain the inability (commonly experienced) to demonstrate physiologically meaningful hormone action in cell-free systems in which the biosynthesis of macromolecules is largely interrupted (*15*).

Table 1. Effect of puromycin and actinomycin on the rise of liver glycogen and tyrosine transaminase levels induced by cortisone. Adult male Sprague Dawley rats starved for 24 hours were injected intraperitoneally with saline suspensions of actinomycin (0.14 mg/100 g) 7 hours before the rats were killed, puromycin (multiple injections, 6 mg/100 g each) at 1, 2, 3, 4, 5, and 6 hours before the rats were killed, and cortisone (25 mg/100 g) 6 hours before the rats were killed. The glycogen content of each liver and the tyrosine-$\alpha$-ketoglutarate transaminase activity [micromoles reaction product, *p*-hydroxyphenylpyruvate (HPP), formed per gram of liver per minute] of three livers in each group were measured (*13*, *14*). Each glycogen value is a mean (± standard error) of results obtained with 14 to 16 separate rats; each of the three tyrosine transaminase values was obtained from a separate liver.

| Substances administered | Glycogen (g/100 g liver) | Tyrosine transaminase ($\mu$mole HPP $g^{-1}$ $min^{-1}$) | | |
|---|---|---|---|---|
| None | 0.32 ± 0.06 | 1.1 | 1.3 | 0.9 |
| Cortisone | 2.56 ± 0.39 | 3.7 | 3.5 | 4.2 |
| Cortisone + puromycin | 0.43 ± 0.07 | 1.4 | 1.5 | 1.8 |
| Cortisone + actinomycin | 0.96 ± 0.14 | 1.7 | 1.6 | 1.4 |


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15. Supported by grants Ca-05034 and Ca-07-37-01 from the U.S. Public Health Service and by the American Cancer Society (E-254).

27 May 1963


## Cerebral Heterostimulation in a Monkey Colony


Abstract. *In an established colony a subordinate monkey repeatedly pressed a lever which stimulated the caudate nucleus of the boss monkey by radio and inhibited his aggressive behavior. In other experiments, timed stimulations of the posteroventral nucleus of the thalamus of the boss monkey, paired with a tone, increased his aggressiveness and established conditioned escape responses of the whole group. Both types of experiments may be useful in neurophysiological and pharmacological investigations.*


Electrical stimulation of some areas of the brain induces positive reinforcement, and rats, cats, and monkeys learn to stimulate themselves by pressing a lever repeatedly for hours or even days (*1*). This method is very valuable for physiological and pharmacological analysis of the central nervous system. Since animals are capable of self-stimulation, they might stimulate the brain of a cagemate if suitable means were provided.

In a colony of four monkeys (*Macaca mulatta*) the boss, Ali (5.2 kg), was an ill-tempered, powerful male who often expressed his aggressiveness by grimacing and biting his own right hand (Fig. 1). Ali had friendly relations with the female, Sarah (4.0 kg), was hostile toward the other female, Elsa (4.6 kg), who ranked No. 3 in the group, and paid less attention to the male, Lou (3.8 kg), who was lowest in social rank, as determined by the peanut test and by offensive-defensive reactions. The colony was housed for several weeks in a cage 7 by 3 by 3