BBA 75292

THE HYDRATIONAL EFFECT OF LEPTAZOL AND ITS THEORETICAL CONNECTION WITH GLUCOSE DEFICIENCY IN THE HAEMOLYSIS OF RABBIT ERYTHROCYTES

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(Received March 3rd, 1969)

SUMMARY

1. The activation parameters for the malonamide-induced haemolysis of rabbit erythrocytes in the presence of leptazol indicate that this drug is an hydration-structure breaker, isokinetic with malonamide and, theoretically, isokinetic with glucose.

2. At concentrations near to those which induce convulsions, the activation parameters for leptazol and glucose are close together, indicating comparable effects on erythrocyte hydration structure.

3. These observations are discussed, and it is suggested that the convulsions induced by leptazol, insulin hypoglycaemia and electric-shock treatment may depend on the disruption of cerebral hydration structure.

INTRODUCTION

A recent study[1] of the effect of barbiturates on the kinetics of malonamide-induced haemolysis of rabbit erythrocytes shows that the activation parameters vary with drug species, and hydrational effects were invoked to account for this. It would seem, if such variation is discernible among drugs of similar biological activity, that greater differences might be expected between drugs with opposing biological effects. We have therefore compared the action of phenobarbitone, a central nervous system depressant, with that of leptazol[2], which is a central nervous system stimulant, and the results are reported below.

MATERIALS AND METHODS

The drugs were compared under conditions identical with those previously described[1], except that rate determinations were made at one osmotic concentration (5.0 atm) only. The test substance, 1,5-pentamethylenetetrazole (Leptazol, Knoll

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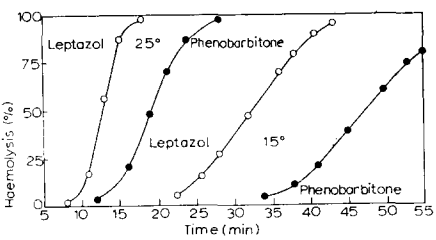
Pharmethicals; British Pharmaceutical Codex, 1963) was present in 0.5 mM concentration.

RESULTS

The typical response depicted in Fig. 1 shows the unequivocal difference in the action of these drugs on the haemolysis of rabbit erythrocytes in hypotonic malonamide of 5.0 atm concentration at 15 and 25°. With leptazol, temperature has a greater effect on the rate of haemolysis—the slope of the curve—than on the lag phase, but with phenobarbitone the converse is true. The activation parameters for leptazol, derived in the same way as before (ref. 1), have the values $\Delta H^{\ddagger} = 20.1$ kcal·mole^{-1} and $\Delta S^{\ddagger} = 50.7$ cal·degree^{-1}·mole^{-1}. The relationship between these and the corresponding values for phenobarbitone, barbitone, amylobarbitone and malonamide alone (control) taken from ref. 1 are illustrated in Fig. 2.



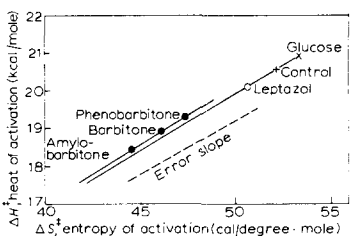


Fig. 1. The effect of 0.5 mM leptazol and phenobarbitone on the haemolysis of rabbit erythrocytes in aqueous malonamide of 5.0 atm osmotic concentration at 15 and 25°.

Fig. 2. The relationship between the activation parameters for haemolysis in 5.0 atm malonamide in the presence of 0.5 mM leptazol (by experiment), 1 mM glucose (by calculation) and 0.5 mM barbiturates (from ref. 1).

The distinction is again clear, because the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for leptazol are not only higher than those for the barbiturates, but they correlate with the control, rather than the barbiturate relationship.

DISCUSSION

The findings substantiate the expectation that drugs with opposing biological activities should differ more in their effect on haemolysis kinetics than those of related activity.

With regard to the activation parameters, the point for leptazol falls on the control curve and slightly below the control value. This means that the basic mechanism of malonamide-induced haemolysis remains unaltered in the presence of the drug, and the lower values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ indicate disruption of total hydration structure. At this concentration therefore, which is above the convulsion threshold (approx. 0.1 mM), leptazol is an hydration-structure breaker.

The correspondence of leptazol with the control (malonamide) has a broader significance, however, because it also implies correspondence with glucose-activation parameters. It has previously been reported[3], with human erythrocytes, that the activation parameters for glucose and malonamide separately, and for glucose and

malonamide together, are collinear, with a common process mechanism that varies only in respect of associated hydrational changes. The same correlation—with the same implications—holds for the activation parameters of malonamide haemolysis of rabbit and human erythrocytes[4], so that the haemolysis of human and rabbit erythrocytes in aqueous solutions of malonamide, glucose and leptazol are isokinetic processes. This is a matter of considerable interest with regard to leptazol because it suggests the possible existence of a physiological connection between the convulsions induced by this drug and those that accompany the glucose deficiency caused by the administration of insulin.

The blood glucose level at the convulsion threshold is about 20–30 mg per 100 ml, which corresponds with a plasma glucose of around 1 mM. From the linear correlations already recorded (refs. 3 and 4), it has been calculated that the activation parameters for the malonamide-induced haemolysis of rabbit erythrocytes in the presence of 1 mM glucose are 20.9 kcal and 53.4 cal·degree^{-1} for $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, respectively. The heat of activation, 5 kcal higher than that for human cells in the same system, reflects the lower rate of haemolysis of rabbit erythrocytes[3]. The graphical location of this calculated point, depicted in Fig. 2, correlates with malonamide and leptazol. The position of leptazol indicates disruption of hydration structure with respect to the control, but with regard to glucose, hydration structure is enhanced. The difference between leptazol and glucose is, however, no greater than that between phenobarbitone and amylobarbitone, which suggests that the biological effects of the first pair could be as closely related.

In these experiments whole blood is diluted in the ratio 1:21 with aqueous malonamide which is 2/3 isotonic, and the resulting cell swelling reduces the structural integrity of total cell hydration. The appearance of leptazol as a structure breaker under these conditions indicates its extreme potency in this respect; in a more ordered (undiluted) system, therefore, this drug is likely to have a relatively greater disruptive action on a weight for weight basis.

Although the same reasoning places glucose in the category of a highly effective structure promoter, it has to be remembered that the concentration discussed here (1 mM) represents the partial replacement only of a glucose deficiency. The physiological level of glucose in rabbit plasma is about 7.4 mM (ref. 5), and with this level of glucose in the haemolysing system, the calculated activation parameters are about 24 kcal for $\Delta H^{\ddagger}$ and 65 cal·degree^{-1} for $\Delta S^{\ddagger}$. An effect of this magnitude at 1/21 dilution is an indication that glucose makes a major contribution to the stability of total cell hydration in the undiluted system. It follows, therefore, that diminishing glucose concentration, whether brought about by dilution or other means, is accompanied by a rapid decrease in the extent and stability of total cellular hydration structure.

According to haemolysis kinetics then, the addition of a convulsant concentration of leptazol creates about the same amount of disorder in the hydration structure of erythrocytes as that equivalent to a deficiency of glucose which is also convulsant.

Although the kinetics and mechanism of erythrocyte haemolysis *in vitro* seem remote from nervous-system physiology, it may be that in terms of hydration, the connection is much closer. It is on record that hypoglycaemic convulsions are highly dependent on the degree of cerebral hydration[6], that dehydration prior to the

administration of insulin prevents these convulsions[7] and that body temperature, which markedly affects hydration, is involved as well[8]. Moreover, in convulsion therapy, which is an established method for treating certain types of mental disease[9], the convulsions may be induced not only by the administration of leptazol or insulin (hypoglycaemia), but also electrically, by the action of a bitemporally applied alternating current[10]. It is not improbable, even in electric convulsion therapy, that hydrational effects are involved, because water molecules are dipolar and their oscillation in an electric field cannot fail to disrupt hydration structure.

In this context the anticonvulsant action of barbiturates may depend on the capacity of their alkyl groups to provide substitute structure in the form of apolar hydration.

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