Non-saturable co-operative binding of tolbutamide to isolated islets of Langerhans

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Functionally active islets of Langerhans isolated from rat pancreas with bacterial collagenase and gathered by means of calibrated sieves, bind tritiated tolbutamide (Alric, Manteghetti, Puech, Lignon & Loubatières, 1975). The bound drug was measured as the amount taken up at equilibrium at 37°C in excess of the [^{14}C]-sucrose space of the preparation. Both isotopes were measured separately by liquid scintillation following combustion of the samples.

The uptake exhibits some features characteristic of binding to cellular structures rather than diffusion to the intracellular space. In particular, tolbutamide uptake shows a non-saturable co-operative pattern in relation to concentrations. The concentration-binding relationships (binding isotherms) show a steadfast upward concavity up to about 5×10^{-5} M, then turn to a straight line.

Such a co-operative pattern indicates that the binding ability of islet cell structures is modified by the binding itself as long as few binding sites are occupied by the drug. This occurs precisely in the range of concentrations which exert a graded stimulating action on the secretion of insulin *in vitro*. At greater concentrations, the binding follows a linear relationship similar to simple physical adsorption on an inert support. Other investigations (Sehlin, 1973) have already shown that this binding finally goes to saturation, but at tolbutamide concentrations far beyond pharmacologically active ones, with a half-maximum at 2×10^{-3} M.

Binding of tolbutamide to exocrine pancreas shows much less co-operativity in the same conditions, indicating a great deal of organ specificity. As was expected, unlabelled tolbutamide 5×10^{-5} M cancels the co-operativity of binding when added just prior to the labelled drug. A similar effect is produced by chlorpropamide and carbutamide at equiactive concentrations (Alric & Portal, 1969) as well as diazoxide at an equi-antagonistic concentration (Loubatières, Mariani, Alric, Chapal & Portal, 1967). But 'second generation' hypoglycemic sulphonylureas glibenclamide and glipizide at equiactive concentrations, and even glisoxepide, in an equimolar amount, failed to do so. Variations of glucose (1 to 16×10^{-3} M) or calcium concentrations did not affect co-operativity of binding.

In spite of the lack of interference of glucose, calcium and second generation sulphonylureas, it remains tempting to consider that the co-operativity of tolbutamide binding to islets could be related to the cell perturbation involved in the stimulating action exerted by this drug on the secretion of insulin by beta cells.

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Interrelations between MAO activity and carbohydrate metabolism

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The possible interrelations between monoamine oxidase (MAO) activity and metabolism of carbohydrates in central and peripheral regions was studied. Ninety per cent inhibition of MAO activity by administration of deprinil (20 mg/kg) in normally fed male rats resulted in 60% decrease in hepatic glycogen whereas cerebral glycogen stores were completely exhausted but the cardiac glycogen rose by 30% Similar administration of deprinil to rats starved for 24 h produced a three-fold increase in hepatic glycogen but cardiac and cerebral glycogen reserves were slightly affected. Activity of enzyme catechol O-methyl transferase (COMT) in the adrenals of fed rats declined by 47% after selective inhibition of MAO by

deprinil but enzyme phenylethanolamine *N*-methyl transferase (PNMT) showed an increase of 20% from non-treated values. Both these enzymes in the adrenals were more severely affected by deprinil in 24 h starved rats since the decrease in COMT and the increase in PNMT were more marked than in the fed animals. Adrenaline stores in the adrenal gland showed an increase but noradrenaline stores declined after deprinil administration to fed rats. The starved rats treated with deprinil demonstrated increased levels of adrenal noradrenaline as well as adrenaline from the value of control rats. Expressing MAO activity per mg of glycogen in each organ and comparing it with MAO per mg of tissue or protein showed direct correlation with variations in glycogen content and MAO activity. The results provide evidence about an important link between the regulatory processes of carbohydrate metabolism and the control of MAO activity in male rats. The use of deprinil also suggests that selective inhibition of MAO activity may have important consequences upon physiological processes of metabolism with respect to glucose and glycogen.

Effects of drugs on suppressed responding

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A type of procedure that has been widely used in studies in behavioural pharmacology has been variously called 'conflict' or 'punishment'. Repetitive responding of the nature of repeated operations of an electrical key by a subject is maintained by a schedule of reinforcement and then, during signalled periods of time, responses are followed by a noxious stimulus according to a program. During the signalled periods, responding is suppressed to a greater or lesser extent, depending on parameter values. This type of procedure has been studied in pigeons, rats and monkeys. There are two main reasons for the popularity of the procedure in pharmacology. First, the results by different investigators from Geller & Seifter (1960) to the present time have been very consistent in showing clearly differential effects of important classes of behaviour-affecting drugs: suppressed responding is increased, i.e. more responding occurs, following effective doses of chlordiazepoxide and barbiturates but not by amphetamine or chlorpromazine which may increase the suppression (Kelleher & Morse, 1968; Dews & DeWeese, 1976). Second, the results lend themselves to plausible descriptions in terms of 'fear' and 'anxiety' that are attenuated by minor tranquillizers.

It has never been demonstrated, however, that the selective effects of the drugs are related to the noxious stimulus (in practice, an electric shock). In the present work responding has been suppressed by a stimulus that is in no way noxious. A rhesus monkey is seated in an isolation chamber with a small lever in comfortable reach in front. When the monkey presses the lever, a white light appears and remains on for 30 s and then goes off. During this period time is being accumulated towards a total interval of between 30 s and 5 min; when that predetermined interval has accumulated, the white light goes out and the next response produces food. The white light therefore merely signals that time is being accumulated toward the moment when food will again be available; nevertheless, the white light suppresses responding. Responding in the presence of the white light is increased by chlordiazepoxide HCl (10–100 μm/kg) and pentobarbital Na (10–30 μM/kg) but not by chlorpromazine HCl (1–10 μM/kg) or methamphetamine HCl (1–10 μM/kg), thus being affected by drugs just like responding suppressed by electric shock. It seems that the selectivity of the drugs depends on the characteristics of the controls of the responding and not on the 'fear' and 'anxiety' engendered by electric shocks and so the commonsense interpretation of 'conflict' and 'punishment' situations is misleading.

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