Influence of betamethasone on concentrations of digoxin in rat serum, liver and heart

P. ATKINSON & J. JACKSON
(introduced by W. C. BOWMAN)

Pharmacia Diagnostics, Hounslow, Middlesex, and Department of Physiology and Pharmacology, University of Strathclyde, Glasgow

It has been demonstrated (Atkinson & Jackson, 1979) that methyl prednisolone can elevate concentrations of digoxin in rat serum and heart. In these experiments bethamethasone, another synthetic glucocorticoid, was administered together with digoxin. The digoxin concentration in the liver was assayed together with the serum and the heart by R.I.A.

Male Sprague-Dawley rats (200 g) were given an intra-muscular injection of digoxin (5 μg) and either betamethasone or saline (the solvent vehicle for betamethasone). After 1 h the animals were killed by a blow on the head and exsanguinated. The blood was centrifuged and the serum was assayed for digoxin. The hearts and livers were dissected out. The organs were sliced and washed three times in Krebs' solution, so that serum would be removed. The organs were then macerated, digoxin was extracted three times with chloroform. The chloroform for each group was pooled and evaporated to dryness. The digoxin was re-dissolved in 6 ml of rat serum, which contained no digoxin. Serial dilutions of this serum were made, so that the assay value would fall on the standard curve.

There were six animals in each treatment group.

The digoxin values for serum, liver and heart are expressed in nM/l and are shown on Table 1.

These results show that betamethasone elevated concentrations of digoxin in rat serum and heart. Betamethasone also lowered concentrations of digoxin in rat liver. This may have been because of competition at non-specific binding sites. This competition may have resulted in the elevated serum concentrations, which would have in turn elevated the concentration in the heart. This study involved serum concentrations greatly in excess of those found in humans undergoing digoxin therapy. The concentrations of betamethasone were also well above those used in therapeutics. This probably means that this interaction would not normally cause problems in patients receiving digoxin (Doherty, 1973).

Table 1 Digoxin concentrations in rats treated with betamethasone

Dose of Betamethasone (mg)	*Concentration of digoxin* (nM/l) ($n = 6$)		
	Serum ± *s.d.*	*Heart*	*Liver*
0	12.1 ± 0.8	5.1	72.3
0.1	12.3 ± 0.9	7.2	63.4
0.5	13.3 ± 1.3	11.2	42.7
1.0	14.6 ± 2.0	16.5	35.4
5.0	15.7 ± 3.2	22.7	36.2

References

ATKINSON, P. & JACKSON, J. (1979). Elevation of serum and heart digoxin levels in rat by methyl prednisolone. *J. Pharm. Pharmac.* (in press).

DOHERTY, J.E. (1973). Digitalis glycosides: Pharmacokinetics and their clinical implications. *Ann. Intern. Med.* **79**, 229–238.

Comparison of individual and cumulative dose-response curves

A. DUNNE (introduced by P.J. CANNON)

Department of Pharmacology, University College Dublin, Belfield, Dublin, 4

The individual dose (I.D.) and cumulative dose (C.D.) techniques for determination of the dose-response curve for histamine on guinea pig ileum are compared. The C.D. determinations were made with 15 s, 30 s and 45 s between doses. Each of four strips of ileum was exposed to all four methods (three C.D. and one I.D.) in a 4 × 4 Latin square arrangement. The resulting sixteen dose-response curves were fitted singly to the equation

$$y = \alpha.\left[1 - \left(1 + \left(\frac{x}{\gamma}\right)^{\beta}\right)^{-1}\right]$$

where

- y = response in arbitrary units.
- x = concentration of histamine in organ bath (0.3 μM to 80 μM).
- α = response at infinite dose.
- β = exponent or 'slope factor'.
- γ = dose corresponding to 50% of the response at infinite dose.

A nonlinear regression computer program was used to obtain the least squares fit. Thus three 4 × 4 Latin squares, corresponding to the three parameters of the model were obtained and analysed using analysis of variance and student-t tests. The main difference between the I.D. and C.D. techniques is shown to be the estimates of the dose corresponding to half the maximal response which are significantly different ($P < 0.01$) for all three C.D. methods investigated. The estimates of the slope factor show no significant ($P > 0.05$) differences between the methods used whilst the estimate of the maximal response is significantly different ($P < 0.05$) only for the C.D. method with 45 s intervals between consecutive doses.

References

VAN ROSSUM, J.M. & VAN DEN BRINK, F.G. (1963). Cumulative dose-response curves I. *Arch. int. Pharmacodyn.*, **143**, 240–247.

VAN ROSSUM, J.M. (1963). Cumulative dose-response curves II. *Arch. int. Pharmacodyn.*, **143**, 299–330.

THE STAFF OF THE DEPT. OF PHARMACOLOGY (EDINBURGH). (1968). Pharmacological experiments on isolated preparations. E. & S. Livingstone Ltd. Edinburgh.

RODBARD, D. & HUTT, D.M. (1974). Statistical analysis of radioimmunoassays and immunoradiometric (labelled antibody) assays. *Radioimmunoassays and related procedures in medicine. (International atomic energy agency, Vienna)*, **1**, 165–189.

DE LEAN, A., MUNSON, P.J. & RODBARD, D. (1978). Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am. J. Physiol.*, **235**, E97–E102.

Prejunctional clonidine-adenosine interactions in the rat vas deferens

K. CALLANAN & A.K. KEENAN

Department of Pharmacology, University College Dublin, Belfield, Dublin, 4

There is now extensive evidence for the role of ATP as a transmitter substance in purinergic nerves and other adenine nucleotides including adenosine have been shown to modulate transmitter release from noradrenergic nerve endings (Burnstock, 1978). In particular, adenosine has been found to inhibit evoked [^{3}H]-noradrenaline release from the pre-labelled rat vas deferens in a dose-dependent manner (Wakade & Wakade, 1978). Furthermore, its depressant action on central neurones is reportedly antagonised by 2-substituted imidazolines including phentolamine and clonidine (Stone & Taylor, 1978). Since the latter is a potent agonist on presynaptic α-adrenoceptors, it was decided to investigate the possibility that the clinidine-mediated α-effect is complicated by antagonism of adenosine.

Desheathed vasa deferentia isolated from male Wistar rats were mounted vertically in an organ bath containing magnesium-free Krebs solution, between silver electrodes through which field stimulation was provided continuously (suprathreshold stimulus, 0.17 Hz, 1 ms). Isotonic contractions were recorded and cumulative log-dose response curves to adenosine obtained, response being measured as percentage inhibition of twitch. Twitches were abolished following addition of tetrodotoxin (1.7×10^{-6} M) to the bath.

The addition of clonidine (1.5×10^{-8} M) caused a leftward non-parallel shift of the adenosine dose-response curve without alteration of the maximum, which is compatible with functional synergism as shown by van den Brink (1977). When phenoxybenzamine (5×10^{-6} M) was added to the bath, the control twitch height increased but the percentage response to adenosine was unaffected. However, the leftward shift now obtained with clonidine was found to be parallel and on further increasing phenoxybenzamine concentration (1×10^{-5} M), there was no difference between responses in the presence or absence of clonidine.

It can be inferred from these results that clonidine and adenosine do not interact at the level of a purinergic receptor located on the sympathetic nerve terminal, since the synergistic effect which is observed in the absence of complete α-blockade disappears at the higher dose level of phenoxybenzamine. These findings are contrary to those of Enero & Saidman (1977), who used rat portal vein, but are in agreement with results obtained by Hedqvist & Fredholm (1976) using guinea pig vas deferens. The possibility that the synergism between adenosine and clonidine occurs because both agents limit the calcium available for excitation-secretion coupling is at present under investigation.

This work was supported by the Medical Research Council of Ireland.

References

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptor. In: *Cell Membrane Receptors for Drugs and Hormones; A Multidisciplinary Approach*, ed.