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.

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Prejunctional clonidine-adenosine interactions in the rat vas deferens

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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.

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Urinary magnesium excretion during amiloride administration in saline-loaded rats

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Reduced urinary magnesium excretion was reported after triamterene administration to either normal human subjects (Hänze & Seyberth, 1967) or saline-loaded rats (Ryan & Phillips, 1977). Acute administration of amiloride to congestive heart failure patients receiving frusemide resulted in reduced urinary magnesium excretion and increased plasma and lymphocyte magnesium (Counihan, Dunne, Halley, Ryan & Ryan, 1978). Canrenoate potassium, an aldosterone antagonist, has been reported to exert magnesium-saving properties in patients with liver cirrhosis (Lim & Jacob, 1978). We have studied urinary magnesium excretion in saline-loaded rats during administration of amiloride either alone or in combination with frusemide.

Male Wistar rats (110–465 g) were fasted overnight with water allowed *ad lib.* All animals received an oral load of 2.5 ml of 0.9% NaCl per 100 g body weight. Diuretics were administered in the appropriate saline load. Rats were placed in individual metabolism cages and urine was collected for 6 hours.

Amiloride (2.5 mg/kg) resulted in a significant diuresis ($P < 0.001$) and natriuresis ($P < 0.001$). The amiloride group excreted an average of 148% of the administered sodium load compared to an average of 45% of the administered sodium load excreted by the control group. Both urinary potassium ($P < 0.001$) and magnesium ($P < 0.01$) excretion were significantly reduced in the amiloride group. Frusemide has been previously reported to enhance urinary output of sodium, potassium, magnesium and calcium in saline-loaded rats (Ryan & Phillips, 1977). In the present study, frusemide (40 mg/kg) induced a natriuresis amounting to an average of 235% of the administered sodium load. Combination of amiloride (2.5 mg/kg) with frusemide (40 mg/kg) produced no further increases in either urinary volume or sodium excretion. Administration of amiloride with frusemide did, however, result in significant reductions in urinary excretions of potassium ($P < 0.001$) and magnesium ($P < 0.01$). Urinary calcium excretion ($P < 0.01$) was significantly reduced during amiloride administration in the presence of frusemide. No reduction in urinary calcium was found during amiloride administration alone. This discrepancy may be related to the presence of calcium, as detected by analysis, in the pharmaceutical preparation of amiloride. Hypercalciuria has been reported after amiloride administration in man (Johny, Lawrence & O'Halloran, 1969).

These preliminary investigations indicate that acute amiloride administration either alone or in combination with frusemide can reduce urinary magnesium in saline-loaded rats. However, more detailed investigations are required to determine whether the reduced urinary magnesium excretion results from a direct action of amiloride on the renal handling of magnesium or is related to possible secondary effects of the diuretic such as extracellular volume contraction.

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