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THE EFFECT OF DIPHENIDOL ON OUABAIN CARDIOTOXICITY IN THE CAT

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1 The capacity of diphenidol to influence ouabain-induced cardiotoxicity was studied in anaesthetized cats with and without spinal cord transection.

2 Diphenidol pretreatment increased the lethal dose of ouabain in both intact cats and cats in which the spinal cords had been transected. Diphenidol pretreatment increased the myocardial content of ouabain associated with death in the intact animals, but failed to influence the lethal ventricular concentration in cats with transected spinal cords.

3 The failure of diphenidol to influence tissue thresholds for toxicity in the spinal cat and the equivalence of tissue ouabain requirements for death in spinal cats and diphenidol-treated intact animals, suggest a neural mechanism for the protective effect in intact animals.

4 Ouabain administration prolonged atrio-ventricular conduction time in all animals and diphenidol attenuated this effect. Thus, the influence of both drugs on atrioventricular conduction may not be entirely mediated by central neurones.

Introduction

Diphenidol (α-diphenyl-1-piperidinebutanol), which inhibits the brain stem chemoreceptor trigger zone (Leonard, Fugita, Tedeschi, Zirkle & Fellows, 1966; Small, 1966), has been shown to reverse digitalis-induced ventricular arrhythmias in the dog (Mandel, Hayakawa, Vyden, Carvalho, Parmley & Corday, 1972). The present investigation was undertaken first to establish the prophylactic value of diphenidol in the management of ouabain-induced rhythm disorders and second, to elucidate the nature of this antiarrhythmic effect.

Methods

Experiments were performed on cats of either sex weighing between 1.6 and 2.0 kilograms. Animals were anaesthetized with sodium pentobarbitone (30 mg/kg) injected intraperitoneally. The right femoral artery was cannulated for the determination of arterial pH, P_{CO_2}, and P_{O_2} and of the serum concentration of ouabain. The left femoral artery was cannulated for continuous monitoring of blood pressure by means of a pressure transducer. A bipolar catheter was inserted in the right internal jugular vein and advanced to the right atrium. The blood pressure, the atrial electrogram and surface electrocardiogram (lead II) were recorded continuously on a polygraph. Both femoral veins were exposed for drug administration.

The trachea was cannulated in all animals. When necessary, the lungs were ventilated mechanically with room air, by the use of a constant volume pump (Harvard small animal model). In these animals, respiratory rate and tidal volume were adjusted to maintain arterial blood pH, P_{CO_2}, and P_{O_2} within normal limits for the cat (Fink & Schoolman, 1963). Body temperature was monitored continuously with a rectal thermometer and maintained between 36°–37°C by means of radiant heat. The vagus nerves were crushed and divided bilaterally in the neck in all animals. In some, the spinal cord was cut at the atlanto-occipital junction. At least 2 h was allowed after spinal section before drug administration to ensure that no residual sympathetic neural activity remained. In all experiments, 1 ml of blood was removed at the time of onset of ventricular tachycardia and at death to determine serum concentration of ouabain.

Tritiated ouabain prepared by catalytic exchange was obtained from New England Nuclear Corporation (Boston, Mass). The final product was characterized by both radiochemical and chemical methods as having purity greater than 97%. Lots of 50 μg of tritiated ouabain with a specific activity of 1.0 mCi/50 μg were first diluted with ouabain injection, USP (Eli Lilly and Co.) and subsequently with 0.9% w/v NaCl solution (saline) so that 1 μg/kg (as the octahydrate) body weight could be delivered in 0.2 ml each minute

Table 1 The influence of diphenidol on the dose (μg/kg), serum level (μg/ml), and ventricular content (pmol/mg wet weight) of ouabain associated with the induction of cardiac rhythm disorders in intact cats and cats after spinal cord section (mean ± s.e.)

	Ventricular tachycardia		*Death*		
	Dose	*Serum level*	*Dose*	*Serum level*	*Ventricular content*
Ouabain (intact) (15) (a)	61.3 ± 1.9[c,d,e]	0.053 ± 0.005[c,d,e]	71.9 ± 2.1[c,d,e]	0.058 ± 0.007[c,d,e]	0.933 ± 0.098[c]
Ouabain plus diphenidol (intact) (7)	86.1 ± 5.1[b,e]	0.110 ± 0.011[b,d,e]	114.0 ± 1.6[b,d,e]	0.106 ± 0.012[b,d,e]	1.21 ± 0.040[b]
Ouabain (spinal section) (7)	87.4 ± 3.5[b,e]	0.259 ± 0.033[b,c]	102.8 ± 4.8[b,c,e]	0.352 ± 0.102[b,c]	1.20 ± 0.031[b]
Ouabain plus diphenidol (spinal section) (8)	111.4 ± 8.4[b,c,d]	0.242 ± 0.037[b,c]	134.2 ± 8.6[b,c,d]	0.336 ± 0.035[b,c]	1.27 ± 0.060[b]

a—The numbers in parentheses indicate the number of animals in each group; b—significantly different from ouabain (intact), $P < 0.05$; c—significantly different from ouabain plus diphenidol (intact), $P < 0.05$; d—significantly different from ouabain (spinal section), $P < 0.05$; e—significantly different from ouabain plus diphenidol (spinal section), $P < 0.05$.

by continuous infusion. Some cats received only ouabain, others received diphenidol (Smith, Kline and French Laboratories) 40 μg kg^{-1} min^{-1} and ouabain simultaneously in separate veins. At the time of death, the infusion was stopped, and the left ventricular myocardium was analyzed for ouabain content. Tissue samples were placed in counting vials, weighed and solubilized with Protosol (New England Nuclear Corporation). The vials were placed in a liquid scintillation counter and allowed to stand at 40°C in the dark for 24 hours. Following this period, the tissue radioactivities were counted and externally standardized. In some instances, internal standardization was performed by the addition of standard tritiated toluene and the counting procedure repeated. These methods determine only total tissue radioactivity, i.e., the drug together with its metabolites, if any. Thin layer chromatography was carried out to characterize the radioactivity as described previously (Levitt, Cagin, Somberg, Bounous, Mittag & Raines, 1973).

The significance of the difference between means was determined by Student's *t*-test.

Results

As shown in Table 1, the dose of ouabain needed to produce cardiotoxicity in intact cats simultaneously treated with diphenidol was significantly higher than the dose needed in cats that did not receive diphenidol. Likewise, the dose of ouabain needed to produce ventricular tachycardia and death was higher in spinal-sectioned cats treated with diphenidol than in spinal sectioned cats not given diphenidol. The dose of ouabain required for the lethal event was highest in spinal-sectioned cats treated with diphenidol; intact cats that received diphenidol required a higher dose of ouabain to reach the lethal end point than did spinal-sectioned cats not treated with diphenidol.

At the time of death, the ventricular content of ouabain was significantly lower in the neurally intact group that did not receive diphenidol than in the other three groups; these all had similar ventricular ouabain contents at death. At the time of ventricular tachycardia and death, the diphenidol-treated group of intact cats also had higher serum levels of ouabain than did the intact cats that did not receive diphenidol but a difference of this kind was not observed in the comparison of spinal-sectioned cats (Table 1). Thin layer chromatography showed that more than 98% of the radioactivity present in the heart was ouabain and not its metabolites.

In general, spinal-sectioned cats had lower blood pressures and heart rates than did intact cats. Diphenidol reduced heart rate before and after the onset of ventricular tachycardia in both intact and spinal-sectioned groups (Table 2).

Table 2 The influence of ouabain with and without diphenidol on heart rate (beats/min), blood pressure (mmHg) and PR interval (ms) in intact cats and cats after spinal cord section

Heart rate	*Control*	*1 min before ventricular tachycardia*	*1 min after ventricular tachycardia*
Ouabain (intact) (a)	215 ± 8[d,e]	206 ± 7[c,d,e]	208 ± 11[c,d,e]
Ouabain plus diphenidol (intact)	195 ± 8[d,e]	180 ± 9[b,d,e]	173 ± 9[b,d,e]
Ouabain (spinal section)	139 ± 7[b,c]	116 ± 8[b,c,e]	133 ± 7[b,c,e]
Ouabain plus diphenidol (spinal section)	132 ± 6[b,c]	90 ± 9[b,c,d]	106 ± 8[b,c,d]
Blood pressure			
Ouabain (intact)	124/95 ± 7/7[d,e]	121/82 ± 11/10[d,e]	112/80 ± 13/11[d]
Ouabain plus diphenidol (intact)	121/91 ± 10/11[d,e]	101/64 ± 9/11[d,e]	96/65 ± 10/9[d,e]
Ouabain (spinal section)	64/38 ± 10/7[b,c]	39/22 ± 9/8[b,c]	39/22 ± 9/10[b,c]
Ouabain plus diphenidol (spinal section)	58/36 ± 8/9[b,c]	40/20 ± 10/9[b,c]	32/18 ± 9/7[b,c]
PR interval			
Ouabain (intact)	67 ± 3[d,e]	87 ± 2[c,d,e]	—
Ouabain plus diphenidol (intact)	67 ± 2[d,e]	74 ± 2[b,d,e]	—
Ouabain (spinal section)	76 ± 3[b,c]	144 ± 9[b,c,e]	—
Ouabain plus diphenidol (spinal section)	75 ± 3[b,c]	113 ± 7[b,c,d]	—

a—The number of cats in each group is the same as in Table 1; b—significantly different from ouabain (intact), $P < 0.05$; c—significantly different from ouabain plus diphenidol (intact), $P < 0.05$; d—significantly different from ouabain (spinal section), $P < 0.05$; e—significantly different from ouabain plus diphenidol (spinal section), $P < 0.05$.

In both spinal-sectioned and intact cats, diphenidol significantly decreased ouabain-induced prolongation of the PR interval (Table 2). Three spinal-sectioned cats not treated with diphenidol had a transient episode of 3° atrioventricular block just before the onset of ventricular tachycardia but the spinal-sectioned cats that received diphenidol did not show 2° or 3° atrioventricular block before the onset of ventricular tachycardia.

All intact cats died in ventricular fibrillation. One spinal-sectioned cat given ouabain alone and one given ouabain and diphenidol died in ventricular fibrillation; the remainder died in asystole.

Discussion

The administration of diphenidol to intact cats increased the dose and serum level of ouabain needed to produce ventricular tachycardia and death and increased the myocardial ouabain content associated with death. The present findings also corroborate the previously reported observation that transection of the spinal cord increases the dose, serum level and ventricular tissue content of digitalis glycosides associated with cardiotoxicity (Levitt *et al.*, 1973; Cagin, Somberg, Bounous, Mittag, Raines & Levitt, 1974). The administration of diphenidol to spinal-sectioned cats further increased the dose of ouabain needed to produce ventricular tachycardia and death. However, the lethal arrhythmia in diphenidol-treated spinal-sectioned cats was not associated with higher ventricular contents and serum levels.

The reason for the dose but not the ventricular content associated with the lethal end point being higher in spinal-sectioned cats given diphenidol may be the slower heart rate observed after this drug during both sinus rhythm and ventricular tachycardia, observations in keeping with previous findings *in vitro* (Mandel *et al.*, 1972; Hayakawa & Mandel, 1973) and *in vivo* (Mandel *et al.*, 1972). That this might be the reason is supported by the finding that the myocardial uptake of digitalis has been shown to vary directly with heart rate (Su, Grupp & Farr, 1972). On the other hand, whereas the slower heart rate in intact cats given diphenidol and ouabain compared to intact cats that received only ouabain may have contributed to the higher dose of ouabain needed to produce death, it does not explain the higher ventricular ouabain content in the intact diphenidol group.

Mandel *et al.* (1972) showed that diphenidol is effective in reversing digoxin-induced ventricular tachyarrhythmias. Our data show that diphenidol is also effective in preventing ouabain toxicity in cats. However, the protection produced by diphenidol is actually no greater than the protection produced by spinal cord transection alone, in the sense that the administration of diphenidol to spinal-sectioned cats did not increase the lethal ventricular tissue content of ouabain.

Digitalis glycosides decrease conduction through the atrioventricular conducting system. Some observers have found that digitalis has no effect on atrioventricular conduction in neurally deprived animals (Schaal, Sugimoto, Wallace & Sealy, 1968; Hirshfeld, Sohn, Raines & Levitt, 1971; Goodman, Rossen, Cannom, Rider & Harris, 1975) but the slowing of conduction by ouabain in our neurally deprived cats is consistent with the observations of Mendez & Mendez (1953).

Diphenidol has been shown to abolish the enhanced spontaneous rhythmicity in dog Purkinje fibres induced by ouabain *in vitro* and thus, is able to oppose toxicity in the absence of a nervous system. However, this phenomenon was observed at low perfusate potassium concentrations (2.7 mEq/l) (Hayakawa & Mandel, 1973) and our findings suggest that diphenidol exerts its principal anti-arrhythmic effects *in vivo* by inhibiting neural influences.

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