J. Pharm. Pharmacol. 1987, 39: 29–34
Received April 2, 1986



# Central dopamine receptors and their role in digoxin-induced cardiotoxicity in the dog

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The role of the dopaminergic system in digoxin-induced cardiotoxicity has been examined. Specific dopaminergic agonists and antagonists were administered into the ventriculocisternal system of pentobarbitone-anaesthetized dogs before systemic administration of digoxin. Pretreatment with apomorphine, a specific dopamine agonist, did not significantly alter the arrhythmogenic or lethal doses of digoxin. However, the digoxin-induced increase in CSF noradrenaline was decreased significantly in apomorphine-pretreated animals. Pretreatment with pimozide, a specific dopamine antagonist, significantly decreased the arrhythmogenic dose of digoxin but did not alter the lethal dose. As with apomorphine, pimozide-pretreated animals accumulated significantly less noradrenaline in CSF compared with control dogs. These results suggest that dopamine receptors are not directly related to the cardiotoxic actions of digoxin. However, dopaminergic receptors may influence the balance of central catecholaminergic systems that influence the peripheral cardiovascular system.

Many studies have supported a role for the central nervous system in digitalis toxicity (Gillis & Quest 1980). Digitalis-induced arrhythmogenesis has been associated with increased sympathetic nerve activity (Gillis et al 1972) and produced through electrical stimulation of the brainstem (Evans & Gillis 1975). Protection against digitalis toxicity has been shown in studies where removal of CNS regulation of cardiac function delays the onset of cardiotoxicity (Helke et al 1979; Somberg & Smith 1979). Pretreatment with autonomic nervous system depressants such as 6-hydroxydopamine, reserpine, and bretylium also prolong the time to arrhythmogenesis (Helke et al 1979; Lathers et al 1981). All of these studies link CNS activation with digitalis toxicity.

Other researchers have linked specific neurotransmitters with digitalis cardiotoxicity. Helke et al (1979) demonstrated an increased noradrenaline turnover in brain which was associated with digitalis-induced arrhythmias. Tackett & Holl (1980) indicated that histamine modulation of the catecholaminergic system may also play a role in digitalis toxicity. The evidence for a dopamine component in the production of arrhythmias was provided by Helke & Gillis (1978) where dopamine agonists increased the doses of deslanoside necessary to produce ventricular arrhythmias and fibrillation. The mechanism proposed was a reduction in central sympathetic outflow. However, none of these studies followed central neurotransmitter changes throughout the course of digitalis administration, or linked cardiotoxicity with in-vivo alterations of neurotransmitter levels. The present study was designed to explore further the role of the dopaminergic system in digoxin-induced cardiotoxicity through the use of specific dopamine agonists and antagonists and by following changes in central neurotransmitter concentrations from initial glycoside administration through arrhythmogenesis to death.

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## METHODS

### *General methods*

Adult mongrel dogs (10–20 kg) of either sex were anaesthetized with sodium pentobarbitone (30 mg $kg^{-1}$, i.v.). After induction of anaesthesia, the animals were intubated and artificially respired with room air. Body temperature was monitored by a temperature probe inserted into the oesophagus (Yellow-Springs) and maintained between 37–39 °C by a heating pad. A femoral artery and vein were cannulated for blood pressure recording and drug administration, respectively. A bilateral vagotomy was performed to remove any reflex vagal responses to the heart. The right jugular vein was cannulated for blood sampling. Cardiac rate and rhythm were monitored by a lead II ECG. All dogs received a continuous i.v. infusion of digoxin (2·5 μg $kg^{-1}$ $min^{-1}$) at a rate of 0·23 mL $min^{-1}$. Doses of digoxin at which sustained arrhythmia and death occurred were noted. Sustained arrhythmia was defined as the presence of idioventricular beats for at least 1 min and characterized by independent non-conducted atrial activity.

*Ventriculocisternal perfusion*

The dog's head was placed in a David-Kopf stereotaxic instrument and cannula inserted into the left lateral ventricle, employing the coordinates provided by the atlas of Dua-Sharma et al (1970). The cisterna magna was approached through the cervical region and cannulated with polyethylene tubing. Artificial cerebrospinal fluid (CSF) (Merlis 1940) was perfused (i.c.v.) at a rate of 0·23 mL $min^{-1}$ throughout the procedure. CSF samples were collected every 15 min in chilled tubes containing glutathione (60 mg $mL^{-1}$) as a preservative. A 60 min stabilization period preceded digoxin administration. The effects of the dopaminergic agents on digoxin cardiotoxicity were determined by administering the drug 60 min before digoxin infusion.

*Digoxin levels*

CSF samples were assayed for digoxin concentrations by radioimmunoassay (Yalow & Berson 1971) which has a calculated sensitivity of 0·17 ng $mL^{-1}$.

*Catecholamine levels*

CSF catecholamine concentrations were measured by HPLC with electrochemical detection. Catecholamines were first extracted on alumina by the method of Anton & Sayre (1962) which has an average recovery of 60% of the catecholamines. The system used was a Beckman 112 solvent delivery system with a BAS-LC 4A electrochemical detector equipped with an Altex ultrasphere C-18 5 μ reverse phase column. The mobile phase consisted of 0·035 M $KH_2PO_4$, 0·015 M citric acid, 1·5 mM sodium octyl sulphate, 2·0 mM $Na_2EDTA$ and 10% methanol, pH 4·85. The flow rate was 1·5 mL $min^{-1}$, The electrochemical detector was set on a sensitivity of 0·5 nA $V^{-1}$, and a potential of 0·55 V. This allowed for analysis of catecholamines with a detection limit of approximately 50 pg injected per sample (signal/noise approximately 2). The retention times of noradrenaline, adrenaline and dopamine were 6, 8 and 25 min, respectively.

*Drugs*

Drugs used were sodium pentobarbitone, digoxin and apomorphine (Sigma Chemical Company, St Louis, MO), and pimozide (Janssen Pharmaceutica Inc., New Brunswick, NJ). Digoxin was dissolved in a solution of 10% ethanol, 40% propylene glycol, and deionized water. Pimozide was dissolved in a 50% solution of ethanol and water. Apomorphine was dissolved in artificial cerebrospinal fluid.

*Statistical analysis*

All data are presented as the mean ± s.e.m. and were analysed using a paired *t*-test or analysis of variance and Duncan's new multiple range test (Steele & Torrie 1960). The criterion for significance was $P < 0.05$.

## RESULTS

*Effects of peripherally administered digoxin*

Cardiac rhythm and arterial blood pressure were monitored in dogs during ventriculocisternal perfusion of artificial CSF and i.v. administration of digoxin (2·5 μg $kg^{-1}$ $min^{-1}$). Cardiotoxicity, manifested as ventricular arrhythmias, occurred at 165 ± 11 μg $kg^{-1}$ of digoxin infused and death at 258 ± 13 μg $kg^{-1}$ (Fig. 1). Death was by ventricular fibrillation in all animals. At the time of toxicity a significant amount of digoxin was present in CSF as shown in Table 1. In another group of dogs (n = 3), the digoxin vehicle (40% propylene glycol, 10% ethanol and deionized water) was administered i.v. at a rate of 0·23 mL $min^{-1}$ for 3 h, to rule out any effects due to the vehicle. No significant changes in blood pressure or heart rate were noted.

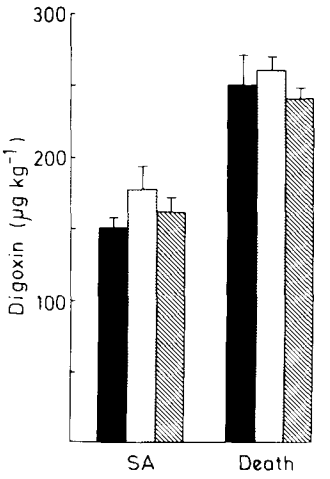


FIG. 1. The doses of intravenous digoxin (2·5 μg $kg^{-1}$ $min^{-1}$ at 0·23 mL $min^{-1}$) to sustained arrhythmias and produce death in dogs infused with digoxin alone (n = 10, black) and following apomorphine pretreatment (70 μg $kg^{-1}$, i.c.v., n = 5, dotted; 300 μg $kg^{-1}$, i.c.v., n = 4, hatched).

Peripheral digoxin infusion led to significant increases in CSF noradrenaline levels before arrhythmogenesis (Table 2). Before digoxin infusion, CSF noradrenaline concentrations were 0·296 ± 0·047 ng $mL^{-1}$ and remained stable until the onset of toxicity. During the 15 min collection period

Table 1. CSF concentrations of digoxin (ng $mL^{-1}$) at the onset of toxicity (arrhythmogenesis) and at death.

| Treatment | Arrhythmogenesis | Death |
|---|---|---|
| Digoxin alone | 4·72 ± 1·72 | 6·09 ± 0·45 |
| Pimozide + digoxin | 4·30 ± 0·52 | 4·92 ± 0·81 |
| Apomorphine + digoxin[a] | 5·21 ± 0·45 | 6·12 ± 0·33 |

[a] Values represent the pooled values of all animals at each dose level.

Table 2. CSF noradrenaline levels (ng $mL^{-1}$) before digoxin infusion (2·5 μg $kg^{-1}$ $min^{-1}$) and before arrhythomogenesis in dogs.

| Treatment | n | Before digoxin infusion | Before arrhythmogenesis | Change (%) |
|---|---|---|---|---|
| Digoxin alone | 10 | 0·296 ± 0·047 | 1·329 ± 0·388[a] | 373 ± 81 |
| Pimozide (500 μg i.c.v.) and digoxin | 5 | 0·226 ± 0·051 | 0·348 ± 0·031[a,b] | 103 ± 32[b] |
| Apomorphine (70 μg $kg^{-1}$ i.c.v.) and digoxin | 5 | 0 152 ± 0·029 | 0·293 ± 0·048[a,b] | 109 ± 29[b] |
| Apomorphine (300 μg $kg^{-1}$ i.c.v.) and digoxin | 4 | 0·157 ± 0·040 | 0·289 ± 0·034[a,b] | 111 ± 37[b] |

[a] $P < 0·05$, significantly different from before digoxin infusion.
[b] $P < 0·05$, significantly different from animals given only digoxin.

preceding arrhythmogenesis, noradrenaline levels had increased to 1·329 ± 0·388 ng $mL^{-1}$ ($P < 0·05$). Dogs infused with the digoxin vehicle showed no significant alterations in CSF noradrenaline over time (3 h) with concentrations of 0·326 ± 0·063 ng $mL^{-1}$. CSF dopamine and adrenaline were not detectable in the CSF in these dogs under the conditions of our assay.

*Effects of centrally administered dopamine agonists and antagonists*

Apomorphine, a specific dopamine agonist, did not significantly alter haemodynamic parameters upon administration into the lateral ventricle (Table 3). Fig. 1 depicts the effects of apomorphine pretreatment on the cardiotoxic actions of digoxin. The dopamine agonist had no significant effects on the arrhythmogenic or lethal doses of digoxin at either dose level (70 and 300 μg $kg^{-1}$). Table 1 illustrates that before arrhythmogenesis, a significant amount of digoxin accumulated in CSF and the level was not different from those values in control animals. All animals in these treatment groups died of ventricular fibrillation. Noradrenaline levels increased from 0·152 ± 0·290 before digoxin infusion to 0·293 ± 0·048 ng $mL^{-1}$ before arrhythmia development in dogs given 70 μg $kg^{-1}$, i.c.v. of apomorphine (Table 2). At the higher dose (300 μg $kg^{-1}$) CSF noradrenaline levels increased from 0·157 ± 0·040 to 0·289 ± 0·034 ng $mL^{-1}$. Thus, the two doses produced a similar magnitude of noradrenaline increase in CSF before the development of arrhythmias. Apomorphine itself had no significant effect on any of the catecholamines during the 60 min pretreatment period. However, there was a significant difference in the actual amount of noradrenaline accumulating in CSF between dogs pretreated with apomorphine and those receiving no pretreatment. The CSF noradrenaline levels increased by 93% (with 70 μg $kg^{-1}$, i.c.v.) and 85% (with 300 μg $kg^{-1}$, i.c.v.) 15 min before arrhythmogenesis in dogs pretreated with the dopamine agonist while the average increase was 349% in dogs given only digoxin (Table 2).

Pimozide, a specific dopamine antagonist, was administered as a bolus (500 μg, i.c.v.) 60 min before digoxin infusion. The only significant change in haemodynamic parameters was a small decrease in heart rate, which was still evident when digoxin infusion was initiated (Table 3). Fig. 2 illustrates that pimozide (500 μg, i.c.v.) significantly decreased the dose of digoxin to ventricular arrhythmias but did not alter the lethal dose of digoxin. CSF digoxin concentrations indicate that significant amounts of drug had accumulated in the CNS before arrhythmogenesis (Table 1). Death in all animals was also due to ventricular fibrillation. The pattern of CSF nora-

Table 3. Effects of i.c.v administered dopamine agonists and antagonists on mean arterial pressure (MAP) (mmHg) and heart rate (HR) (beats $min^{-1}$) in dogs.

| Pretreatment | n | Before pretreatment MAP | Before pretreatment HR | 60 min before treatment MAP | 60 min before treatment HR | Maximal effects MAP | Maximal effects HR |
|---|---|---|---|---|---|---|---|
| Pimozide (500 μg i.c.v.) | 5 | 147 ± 6 | 163 ± 9 | 142 ± 7 | 151 ± 9* | −3 ± 6 | −9 ± 5* |
| Apomorphine 70 μg $kg^{-1}$ i.c.v. | 5 | 146 ± 4 | 153 ± 7 | 138 ± 4 | 147 ± 4 | −7 ± 11 | −2 ± 9 |
| 300 μg $kg^{-1}$ i.c.v. | 4 | 134 ± 14 | 161 ± 13 | 135 ± 12 | 166 ± 17 | −6 ± 13 | 6 ± 8 |

*$P < 0·05$, significantly different from before pretreatment.

drenaline increases before arrhythmogenesis was the same as with apomorphine pretreatment (Table 2). Noradrenaline increased from 0·226 ± 0·051 ng mL$^{-1}$ before digoxin infusion to 0·348 ± 0·031 ng mL$^{-1}$ before ventricular arrhythmias. Neither pimozide nor its vehicle significantly affected CSF noradrenaline levels over a 1 h pretreatment period. Like apomorphine, pimozide-pretreated animals accumulated significantly less noradrenaline in CSF before arrhythmogenesis compared with control dogs. A 349% increase of CSF noradrenaline levels occurred 15 min before arrhythmogenesis in control dogs while the average CSF noradrenaline level increased by only 54% in dogs pretreated with pimozide (Table 2).

*Effects of peripherally administered pimozide*

To ensure that the effects produced by central administration of pimozide were due specifically to central mechanisms, a group of dogs was pretreated peripherally with pimozide (500 μg, i.v.) 60 min before digoxin infusion. The bolus dose of pimozide did not significantly alter haemodynamic parameters, and the doses of digoxin to toxic and lethal levels were not altered over animals given digoxin alone (Fig. 2).

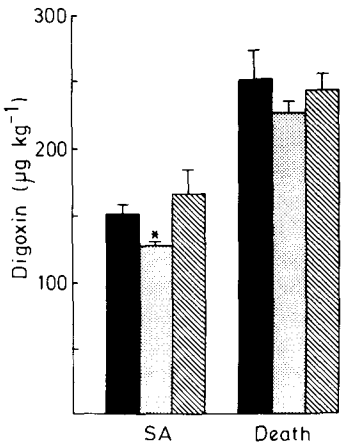


FIG. 2. The effects of pimozide pretreatment (500 μg i.c.v., n = 5, dotted; 500 μg i.v., n = 3, hatched) on the doses of digoxin (2·5 μg kg$^{-1}$ min$^{-1}$ i.v., black at 0·23 mL min$^{-1}$) to sustained arrhythmias and death in dogs.

## DISCUSSION

The results of the present study do not support the direct involvement of central dopaminergic receptors in digoxin-induced cardiotoxicity in the dog. Pretreatment with the dopamine agonist apomorphine did not alter digoxin cardiotoxicity (Fig. 1). Pimozide, a specific dopamine antagonist, decreased the arrhythmogenic dose of digoxin but did not change the lethal dose of the drug compared with dogs given digoxin alone (Fig. 2). Peripheral administration of pimozide did not alter the arrhythmogenic or lethal doses of digoxin, ruling out any peripheral contributions to the actions of pimozide. The lack of an effect by apomorphine and the inability of pimozide to increase the lethal dose of digoxin indicate that dopaminergic receptors are not directly linked to the development of digoxin cardiotoxicity.

These results are contrary to the findings of Helke & Gillis (1978) who showed that centrally administered apomorphine increased the cardiotoxic and lethal doses of deslanoside in cats. Further, haloperidol, a non-specific dopaminergic antagonist, prevented the protective actions of apomorphine but had no significant effect itself on the doses of deslanoside required to produce ventricular arrhythmias and ventricular fibrillation. The effects of the dopaminergic agents were attributed to central activation of dopamine receptors with a consequent decrease in central sympathetic outflow. The lack of an effect by apomorphine in our study may be due to species differences. This possibility has been associated with the actions of β-adrenoceptor antagonists in digitalis toxicity (Cheymol et al 1972). Many other drugs have also been shown to exhibit species-specific activity. The present study used doses of apomorphine equivalent to those of Helke & Gillis (1978), as well as a larger dose (300 μg kg$^{-1}$ i.c.v.). However, 70 μg kg$^{-1}$ i.c.v. of apomorphine did not significantly alter blood pressure or heart rate in the dog, while Helke reported significant depressor responses upon apomorphine administration into the lateral ventricle in the cat. Further, the larger dose of apomorphine also failed to alter haemodynamic parameters. This indicates that differential responses in the dog versus the cat may occur with central apomorphine administration.

The effects of pimozide in our study are also contrary to the findings of Helke & Gillis (1978). They used haloperidol, a non-specific antagonist, which has both α- and dopamine receptor antagonist activity in the CNS (Janssen 1967). Pimozide is more selective for dopamine receptors and has little if any α-antagonist activity (Janssen 1967; Andén et al 1970). Therefore, although the anti-apomorphine effects of haloperidol reported by Helke & Gillis (1978) may be due to dopamine receptor antagonism, the lack of an effect by haloperidol on the

cardiotoxic or lethal doses of deslanoside may be attributed to α-adrenergic activity. Plunkett & Tackett (1983) have shown that α-receptor blockade provides protection against digitalis toxicity while α-receptor stimulation enhances toxicity. Therefore, the combined actions of haloperidol on α-receptors and dopamine receptors may be opposing in their effects as evidenced by the enhanced arrhythmogenic effects of digoxin shown with pimozide in our study.

In all of the groups, we observed increases of noradrenaline before arrhythmogenesis, with no detectable changes in dopamine or adrenaline levels in CSF. However, apomorphine and pimozide pretreatment reduced the amount of noradrenaline released by digoxin (Table 2). This substantiates the idea that digitalis produces an increased sympathetic discharge in the CNS through the release of some neurohumour (Saxena & Bhargava 1975) and further confirms the findings of Plunkett & Tackett (1986). Diffuse central activation following neurotransmitter release may then predispose the heart to cardiac arrhythmias by producing enhanced, but discordant, neural influences to the myocardium (Lathers et al 1981). Helke et al (1979) provided evidence for the role of noradrenaline in the production of arrhythmias by digitalis; an increased level of 4-hydroxy-3-methoxyphenylglycol (MHPG) was found in the brain tissue of animals killed immediately after arrhythmia development. Our data directly link noradrenaline with arrhythmogenesis through in-vivo sampling of CSF and the observation of increased noradrenaline levels immediately preceding ventricular arrhythmias.

The effects of the dopaminergic agents on the degree of accumulation of noradrenaline in CSF indicates that central dopamine activity may modulate the enhanced sympathetic activity associated with digoxin cardiotoxicity. The dopamine system in the CNS has been associated with decreased sympathetic nerve discharge (Laubie et al 1969; Finch & Haeusler 1973). Many studies have suggested that presynaptic dopamine receptors can inhibit transmitter release from noradrenergic nerve terminals and thus decrease sympathetic responses (Rand et al 1975). An intact dopamine system in the brain could act to counter the neuroexcitation associated with digitalis. Thus, our data, showing significantly increased noradrenaline but reduced accumulation in CSF before arrhythmogenesis in apomorphine-pretreated dogs, support this hypothesis of dopaminergic inhibition of sympathetic activity.

The effects of pimozide on digoxin cardiotoxicity and the degree of CSF noradrenaline accumulation are not as clearly defined. Pimozide, a selective dopamine antagonist, decreased the arrhythmogenic dose of digoxin (Fig. 2) but also decreased the amount of noradrenaline accumulated in CSF before arrhythmogenesis (Table 2). This drug is one of the most selective dopaminergic antagonists and is a potent stimulator of dopamine turnover, decreasing endogenous dopamine levels, but it has minimal effects on endogenous noradrenaline turnover (Pinder et al 1976). Pimozide would be expected to enhance stimulated release of noradrenaline by disrupting the inhibition of release through the presynaptic dopamine receptor. However, our results show the opposite effect, a decreased level of noradrenaline in CSF. This indicates that other unidentified factors in addition to dopamine autoreceptor activity may be responsible for the decreased degree of noradrenaline accumulation in CSF after either dopamine agonist or antagonist administration.

The actions of pimozide in enhancing cardiotoxicity and the lack of an effect by apomorphine in protecting against arrhythmogenesis also suggest that central dopamine receptors are not the direct link to the production of increased sympathetic outflow. Digitalis has been reported to block central dopamine receptors upon intracerebroventricular administration (Doggett 1973). Blockade of central dopamine receptors by digitalis would lead to an increased turnover of dopamine in the brain, which was reported by Helke & Gillis (1978). Pimozide also increases dopamine turnover and thus decreases endogenous neurotransmitter levels (Pinder et al 1976). Thus, pimozide may act to decrease the inhibitory influences on sympathetic nervous system activity in the CNS and enhance any effects associated with digoxin in the CNS. The lack of an effect by apomorphine, however, indicates that central dopamine receptors are not the direct link to the production of increased sympathetic outflow, but instead contribute to the overall actions of digoxin in the brain.

In conclusion, dopamine receptors are not directly related to the production of ventricular arrhythmias by digoxin in the dog. They may have an influence on the balance of the central catecholaminergic systems that influence the peripheral cardiovascular system. In this role, pimozide enhanced the cardiotoxic actions of digoxin by potentiation of the increased sympathetic discharge associated with digoxin toxicity. Increases in CSF noradrenaline levels before arrhythmogenesis were common to all dogs regard-

less of pretreatment, although dopaminergic drugs decreased the amount of noradrenaline accumulating in CSF before toxicity. It is this noradrenergic system that plays the major role in the events leading from arrhythmogenesis to death and may be the initiating factor in digoxin-induced arrhythmogenesis.

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