The Pharmacology and Subacute Toxicology of Dopamine

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It is well known that following trauma, myocardial infarction or major surgery, hæmodynamic imbalances frequently occur which result in decreased perfusion of vital organs. The human body typically attempts to compensate for these imbalances by increased autonomic activity. In some instances the response is appropriate and homeostasis is achieved. However, in some patients, despite correction of pre-existing hypovolæmia, the autonomic discharge produces an inappropriate response leading to inadequate perfusion of the vital core organs, and deterioration to clinical shock. Pharmacological intervention with adrenergic agonists is one of the most frequently used approaches to the therapy of non-hypovolæmic shock. Several agents in this general class are utilized, and an understanding of their rational use devolves upon an understanding of the principal types of receptor activated by each.

The adrenergic agents most frequently used in the United States to correct hæmodynamic imbalances are norepinephrine, dopamine and isoproterenol (Fig 1). Although dopamine has proved to be an important and useful new drug, it must be emphasized that it may not always be the appropriate drug for a particular patient in shock. In some instances better clinical responses may be obtained with norepinephrine, and in others with isoproterenol. The hæmodynamic needs of the patient are the determining factor in the choice of drug. Although all three of these agents appear to be structurally similar, they are rather diverse in their predominant pharmacological activity. Norepinephrine is primarily an alpha adrenergic agonist, and as such produces peripheral vasoconstriction, but it also possesses $beta_1$ activity. Isoproterenol is essentially a $beta_1$ and $beta_2$ receptor agonist.

Pharmacology of Dopamine

Dopamine is unusual among adrenergic agents in that its predominant type of activity is highly dose-dependent and varies somewhat from species to species. The first literature reports, by George Barger and Sir Henry Dale (Barger & Dale 1910), characterized dopamine principally as a pressor amine with a potency 1/50th that of norepinephrine. Tainter (1930) later reported that in cats pretreated with ergotoxine, dopamine at doses which normally were pressor produced a decrease in blood pressure. For some time it was felt that this depressor effect was due to $beta_2$ receptor activation, similar to that elicited by epinephrine.

The first evidence that dopamine produced vasodilation by activation of a unique receptor mechanism was reported by Goldberg and his colleagues (McDonald *et al.* 1963, 1964) who found that intravenous injections of dopamine in normal human subjects produced a marked reduction in renal arterial resistance.

Subsequent papers from Goldberg's group (McDonald & Goldberg 1963, McNay & Goldberg 1966, McNay *et al.* 1963, 1965) confirmed the renal vasodilating effect in dogs and further reported that neither phenoxybenzamine, an alpha-blocking agent, nor DCI, a beta-blocking agent, abolished this effect. In 1964 Eble reported that dopamine dilated the superior mesenteric and cœliac vascular beds by a mechanism apparently identical to that described by Goldberg. This action has been called dopaminergic because it is highly structurally specific. Very few chemical structures unrelated to dopamine appear to possess this activity, and the action is not attenuated by either alpha or beta blockade. Although isoproterenol can increase renal blood flow, this action is abolished by pretreatment with propranolol or other beta blockers having peripheral ($beta_2$ inhibitory) activity.

At somewhat higher doses dopamine exerts inotropic and, to a lesser degree, chronotropic

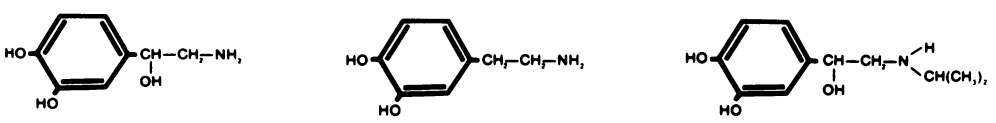

Fig 1 *Catecholamines commonly used in the USA for treatment of shock*

effects on the myocardium, similar to those produced by isoproterenol. These myocardial ($beta_1$ agonist) effects of both agents can be antagonized by propranolol. However, there is a quantitative difference between these two cardioactive agents. The increment in force of contraction or stroke volume produced by dopamine is accompanied by a smaller increase in heart rate than that demonstrable with isoproterenol. This has been reported by Tsai *et al.* (1967) in the isolated guinea-pig heart and by Browne & Zaroslinski (1971) in the isolated rat heart.

Dopamine also has some peripheral $beta_2$ agonist activity, which is demonstrable in the dog only at high doses and in the presence of alpha adrenergic blockade. This activity does not appear to be of therapeutic importance.

High doses of dopamine produce a peripheral alpha response which can mask, or override, dopaminergic vasodilation. At such doses renal blood flow may be diminished to a point at which urine flow virtually stops. This has been reported by Goldberg *et al.* (1969), as well as by Zaroslinski & Browne (1971). Similar results have been reported in human trials. However, judicious simultaneous use of alpha blockers in such patients has been shown to restore the urine flow (Goldberg *et al.* 1969, MacCannell *et al.* 1966).

Direct vs *Indirect Activities of Dopamine*

Catecholamines and sympathomimetic amines in general exert their adrenergic activity in one of two ways, or by a combination of both. They act either directly on the sympathetic receptor site or indirectly on endogenous neuronal catecholamine storage sites to release norepinephrine, or by a combination of both mechanisms. Among these agents there is a continuum of activities ranging from exclusively direct acting (e.g. norepinephrine) to exclusively indirect acting (e.g. tyramine). In the case of dopamine the situation is slightly more complex, due to the precursor-product relationship between dopamine and norepinephrine.

A number of studies designed to characterize the position of dopamine along this direct *vs* indirect spectrum have been reported. In isolated heart preparations from various species, Gurd (1937) demonstrated that pretreatment with cocaine, an inhibitor of norepinephrine uptake, reduced but did not abolish the inotropic action of dopamine. More recently, Tsai *et al.* (1967) reported a reduction in the chronotropic effect of dopamine in isolated guinea-pig hearts from animals pretreated with reserpine, but noted that this decrease in sensitivity to dopamine was only by a factor of 1.45. Farmer (1966) has shown that the chronotropic effect of dopamine in the spinal cat is attenuated, but not abolished, by pretreatment with reserpine or cocaine.

Despite the apparent indirect component of the cardiac effects of dopamine, Tuttle (1970) observed that, for an equal increase in cardiac contractility, dopamine causes less increase in heart rate than most sympathomimetics, but he also noted that the inotropic effect was more attenuated by DMI, an inhibitor of norepinephrine release, than was the chronotropic effect. However, Endoh (1975) has presented data to suggest that the action of dopamine on the sinus node is not fundamentally different from that on papillary muscle, but that their apparently different sensitivities may be related to different vascularization of the two sites.

Although the myocardial effects of dopamine are partially mediated by norepinephrine release, studies with agents interfering with norepinephrine uptake indicate that the vascular effects of dopamine are mediated directly. Browne & Zaroslinski (1971) demonstrated that reserpine did not modify the pressor effects of dopamine in anæsthetized rats, while Tuttle (1975) showed that pretreatment with DMI produced no change in the pressor effect of dopamine in anæsthetized dogs.

The precursor-product relationship between dopamine and norepinephrine in the adrenergic nerve terminal is well known. Hence, in assessing the pharmacological activity of exogenously administered dopamine, consideration must be given to this potential mechanism of indirect activity. Inhibition of the intraneuronal enzyme, dopamine beta-hydroxylase, by means of disulfiram, did not alter the ED_{50} of the dopamine-induced chronotropic effect in isolated guinea pig atria (Tsai *et al.* 1967). In addition, Browne & Zaroslinski (1971) reported no effect of disulfiram on the chronotropic or inotropic effects of dopamine on isolated rat atria. Moreover, these investigators showed no significant effect of disulfiram on the dopamine-induced pressor response in the intact rat *in vivo*. Hence, it would appear that the contribution of exogenous dopamine to *de novo* synthesis and release and to the pharmacological response to dopamine is negligible, but that a portion of the myocardial response to dopamine is indirectly mediated by norepinephrine release.

Acute and Subacute Toxicology of Dopamine

There are few data in the scientific literature on the toxicology of dopamine. However, extensive acute and subacute toxicology studies have been conducted in our laboratories. Table 1 lists the acute LD_{50} data. The Litchfield Wilcoxen method was used for calculations of LD_{50} and

Table 1

Dopamine hydrochloride: acute toxicity

	Route	LD_{50} *(mg/kg)*
Male mice (bodyweight 20–25 g	Oral	2075 (±81.5)
	Subcutaneous	1950 (±133)
	Intraperitoneal	970 (±74.0)
	Intravenous	290 (±14.0)
Male rats (bodyweight 115–145 g)	Oral	2800 (±140)
	Subcutaneous	2575 (±215)
	Intraperitoneal	1015 (±87.5)
	Intravenous	38.8 (±6.15)
		ALD_{50} *mg/kg*
Male rabbits (bodyweight 2.3–2.7 kg)	Intravenous	125–150
Male and female dogs (bodyweight 6–15 kg)	Intravenous	75–100

Figures in parentheses represent 95% confidence limits

95% confidence limits for mouse and rat. Ten animals were used per group, and the observation period was 24 hours. The oral LD_{50} in the mouse was 2075 mg/kg. The 95% confidence limits are shown in parentheses. The intravenous LD_{50} was approximately 290 mg/kg, so that the drug is seven times as toxic intravenously as it is orally. The i.v. LD_{50} of 39 mg/kg in the rat was substantially lower than in the mouse, and this may be because it is less bound to silent receptors. The i.v. approximate LD_{50} (ALD_{50}) in the rabbit was between 125 and 150 mg/kg; in the dog it was between 75 and 100 mg/kg.

Examination of animals which had died disclosed massive internal bleeding, presumably a consequence of this drug's alpha and $beta_1$ adrenergic effects, which obviously would be exaggerated at lethal doses. Evidence of pulmonary congestion was also noted.

Subacute rat toxicity studies were carried out in Sprague-Dawley rats, using ten males and ten females in the control group and in each of the three dose level groups for a period of two weeks. The drug was administered intraperitoneally. The high dose group was given the calculated LD_{50} dose, 570 mg/kg. The other two experimental groups received one-half and one-fourth of the LD_{50} dose respectively. At the end of the study blood was withdrawn from all animals for determinations of total red and white cell counts, differential, hæmatocrit and hæmoglobin. In addition, blood sodium, potassium, BUN, SGOT, prothrombin, and fasting blood sugar were determined. At autopsy the following organs and tissues were removed for gross and histopathological examination: heart, lungs, liver, kidneys, adrenals, thyroid, gonads, spleen, skeletal muscle, thymus, uterus and brain. The first eight of these organs were all weighed. The hæmatology and blood chemistry values did not show any consistent pattern of deviation from generally accepted limits. In a few isolated instances we found artificial deviations, unrelated to drug dose.

Males in the high dose group showed evidence of bilateral hydronephrosis associated with marked bladder distension. Similar changes were not observed in females. Further pathological examination revealed prostatic enlargement, which appeared to be the cause of the bladder distension and hydronephrosis. Further examination of the prostate in lower dose groups showed similar enlargement, but it was less marked. Histopathological examination of the tissue showed marked prostatic glandular dilatation, often associated with inflammation. Similar findings were reported by Farnsworth & Lawrence (1965) after administration of both epinephrine and norepinephrine, at considerably lower doses than those which we employed. It was noteworthy that there was no evidence of damage to the large or small intestine, nor to peripheral skeletal muscle. In the high dose group (570 mg/kg per day) heart, kidney and lung weights were significantly higher than in controls, while the spleen was significantly lighter in weight. Increased heart weight was a consistent finding at all of the doses studied, but it was not associated with any observable histopathological change. The changes were similar in magnitude to those reported by Alderman & Harrison (1971) with isoproterenol.

A second subacute rat toxicity study was carried out using lower doses. At doses of 100 mg/kg per day or less there were no consistent pathological findings attributable to the drug. As in the previous study, both hæmatological values and blood chemistry were within normal limits.

Since dopamine was intended to be administered on a continuous basis, a toxicity study was planned in which the drug would be administered intravenously on a 24 hour basis for two weeks. Fig 2 shows the experimental preparation. Each dog was prepared with a chronic indwelling catheter made of size PE20 silicone tubing, which was passed down the right external jugular vein to the level of the right atrium. The other end was brought up subcutaneously and exteriorized through the skin between the shoulder blades, as shown in the diagram. This exterior portion of the cannula was passed through a hollow steel cable; one end was attached to the dog by means of a harness while the other was attached to a constant infusion pump. The length of the steel cable allowed the animal reasonable freedom from restraint, so that it could sit or lie down in the cage. The high dose group received 37.5 μg/kg per min i.v., and the other groups received one-half and one-fourth of this dose respectively. Each group contained three male and three female pure-bred beagles. Hæmatological tests

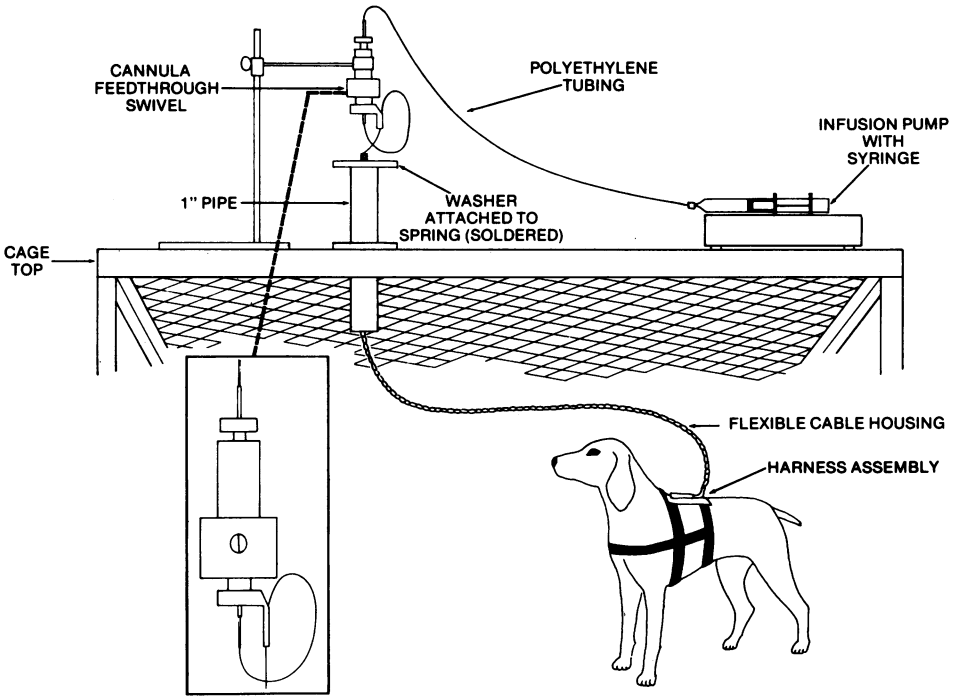

Fig 2 *Diagrammatic representation of dog preparation for subacute fourteen-day continuous infusion of dopamine*

conducted before the study and at autopsy included RBC, WBC and differential count, hæmoglobin, hæmatocrit, clotting time and clot retraction. Included in the blood chemistry tests were levels of sodium, potassium, BUN, SGPT, fasting blood sugar, and alkaline phosphatase. Complete urinalyses were also carried out.

The principal problem encountered with the animals was the repetitive vomiting which afflicted the high dose group. This subsided as the study continued. The animals remained generally in good health, and at autopsy virtually all major organs and tissues were examined macroscopically. In addition histological sections of heart, lung, liver, kidneys, adrenals, thyroid, spleen, gonads, prostate, brain, duodenum, pancreas, urinary bladder, gall bladder, colon, lymph nodes, submaxillary glands, skeletal muscle, and rib plus marrow were examined. The first nine of these tissues were weighed, and the data were analyzed statistically. The adrenal glands of all of the drug-treated groups were significantly heavier than those of controls, a common finding in most toxicity studies. The prostates of the animals in the high dosage group were significantly heavier than those in the control groups. However, in groups three and four, which received 18.75 and 9.375 μg/kg per min, the prostate weights did not differ significantly from controls. Microscopic examination of the prostate did not disclose any pathology. Our pathologist did find small areas of focal necrosis in the myocardial tissue of five out of six animals in the high dose group. He described these as tiny, focal, usually minimal, microscopic in size, and principally limited to muscle cells. We feel that these small areas of focal necrosis can be explained by drug-induced vascular spasm leading to transient tissue hypoxia. They were smaller in size than those reported in the literature for isoproterenol and norepinephrine. In some lesions there were small intramural arteries showing intimal proliferation and perivascular œdema with fibrous proliferation. Focal myocarditis, subendocardial or pericardial hæmorrhage or evidence of heart failure were not seen.

Laboratory values for hæmatology, blood chemistry and urinalysis were in virtually all instances within the ranges for the control group.

Summary

Preclinical studies with dopamine showed a unique spectrum of biological activities which suggested that it might be of therapeutic use in the clinical syndromes of shock and low cardiac output. Most prominent among these were its effects on cardiac output, renal perfusion, and vital organ flow. The unique effect on renal

function and the subsequent studies by Goldberg and his colleagues led to the recognition of a previously unknown catecholamine receptor site, the 'dopaminergic receptor'. Studies on the toxicology of dopamine in our laboratories suggested that dopamine could be safely used in the clinic.

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DISCUSSION

Dr F Hughes (*London*): To what do you attribute the vomiting in the dog after the i.v. injection? Did you investigate that at all?

Dr Zaroslinski: The vomiting, I believe, is due to the same type of activity as is exhibited by apomorphine. Apomorphine is one of the few drugs which exhibits dopaminergic activity, and I strongly suspect that dopamine was stimulating the same vomiting receptor which is sensitive to apomorphine. We find that many compounds with dopaminergic activity do produce vomiting in animals, and some people have been using vomiting as a screening procedure for dopaminergic activity, for trying to identify analogues of dopamine.

Professor Dollery (*Chairman*): Did you measure the creatine phosphokinase in the blood of the animals, the dogs, that were perfused?

Dr Zaroslinski: No, we did not. At the time this procedure was not being used very extensively. It would have been a very useful investigation.