FURTHER STUDIES ON THE SYMPATHOMIMETIC ACTION OF TETANUS TOXIN

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The usual cause of death in tetanus is thought to be exhaustion following repeated convulsions and paralysis of respiratory centre (1-4). However, cardiac arrest, pulmonary oedema, hypertension or hypotension have also been observed and attributed to central or medullary intoxication (1, 5-7). Histopathological and electrocardiographic evidences indicate involvement of the myocardium (8-10). Further, fluctuations in blood pressure and tachycardia often complicate the clinical picture.

Previous investigations carried out in our laboratory had revealed that intravenous injection of tetanus toxin in dogs and rats produces a triphasic response on systemic blood pressure. An initial slight pressor effect is followed by a sharp depressor response which is next followed by a sustained pressor phase. The depressor phase was absent in dogs pretreated with the antihistamine drug, mepyramine and in rats pretreated with cyproheptadine which is both antihistamine and antiserotonin. The delayed pressor phase was absent after treatment with phenoxybenzamine, a drug with α-adrenergic blocking properties. The toxin was also shown to produce a stimulation of the isolated rabbit heart and reduce the coronary flow in the same preparation. It also reduced the flow through isolated perfused rat hind limbs. The toxin mimicked the action of adrenaline on guinea pig vas deferens and this was blocked by phenoxybenzamine. Thus there was a resemblance between the actions of the tetanus toxin and those of adrenaline. Studies were undertaken to assess this relationship further and are now being reported.

MATERIALS AND METHODS

Tetanus toxin: The tetanus toxin obtained from Haffkine Institute, (Bombay) contained 20,000 MLD mice per ml and was preserved at 4°C. The toxin was diluted immediately before use when necessary.

Animals: Mongrel stray dogs were obtained from municipal pounds. Albino rats (150-200 g) of either sex were obtained from the Central Animal House of this institution. The diet for these animals has been described previously (11).

Studies on dog heart in situ: The dogs were anaesthetized with intraperitoneal injection of pentobarbitone sodium (30 mg·kg). The systemic blood pressure was recorded from the carotid artery (12), and the femoral vein was cannulated for intravenous injections. The heart was exposed by splitting the sternum and incising the pericardium. The contractions of the auricle and ventricle were recorded on a smoked drum.
Studies on coronary circulation: The coronary flow was measured in dogs (after exposure of the heart as described above) by cannulating the anterior descending branch of the left coronary artery (13). The flow was measured directly as ml/min, or alternatively measured using Condon’s drop recorder.

Hind limb perfusion of the rat: The hind limbs of the pithed rats were perfused with warm oxygenated Ringer Locke solution at a constant pressure (14). The venous return was measured directly or recorded with Condon’s drop recorder.

Peripheral blood flow in the dog: The dogs were anaesthetized as above and the femoral artery was exposed. The proximal and distal ends of the cut artery were connected with a three way stop cock for giving intraarterial injections. The femoral vein was cannulated to record the flow. A mass ligature was tied at the upper end excluding the femoral artery and the vein. Thus the blood supply to the limb was through femoral artery and drainage only through femoral vein.

The blood sugar levels: The blood sugar levels were determined by standard Folin and Wu technique (15).

Drugs: Phenoxybenzamine (dibenzyline) was used to antagonize α-adrenergic actions. In dogs, the drug was administered in a dose of 5 mg/kg body weight 30 minutes before injection of adrenaline or toxin. The drug was given in 1 mg doses over 5 minutes in rat hind limb perfusion set up.

![Figure 1](image-url)

**Fig. 1.** The effect of tetanus toxin on the blood pressure, auricular (AUR) and ventricular (VENT) contractions of the dog. Tracings from different experiments. The right hand tracing with fast kymograph shows positive chronotropic effect. Blood pressure scale mm of mercury.
Dichloroisopropyl-noradrenaline (DCI) was used in doses of 4 mg/kg body weight intravenously to antagonize $\beta$-adrenergic actions in dogs.

Cocaine was administered in a dose of 1 mg over 5 minutes in a rat hind limb perfusion set up in order to potentiate adrenaline actions.

Reserpine was administered to rats in a dose of 4 mg/kg body weight intraperitoneally, 24 hours before the experiment in order to produce depletion of catecholamine stores (16).

RESULTS

*Effects of tetanus toxin on the dog heart in situ*

When contractions of the auricles and ventricles in situ were simultaneously recorded along with blood pressure, it was seen that the toxin produced marked positive inotropic and chronotropic actions on the heart (Fig. 1). The effects on the heart were instantaneous. These effects were very much reduced or abolished after injection of dichloroisopropyl-noradrenaline (DCI) was used in doses of 4 mg/kg body weight intravenously to antagonize $\beta$-adrenergic actions in dogs.

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![Fig. 2. The effect of tetanus toxin on the blood pressure (BP), ventricular (VENT) and auricular (AUR) contractions of the dog. All three tracings from same animal. The white arrows in the first two tracings indicate the point of injection of tetanus toxin where as the same in last tracing indicates the injection of calcium chloride. Dichloroisopropyl-noradrenaline was injected between the first and second tracings (double arrow).](image-url)
propylnoradrenaline, in doses in which it blocked the actions of adrenaline or isoprenaline but not that of calcium (Fig. 2). It may be seen that a fall in blood pressure occurred in this set up inspite of cardiac stimulation. Similar responses were obtained in all the six experiments performed.

In animals pretreated with phenoxybenzamine, the initial pressor and depressor phases were not affected but the delayed pressor effect was abolished. However, there was no reversal or actions as is seen with adrenaline.

Effects of tetanus toxin on coronary circulation of the dogs

The injection of tetanus toxin produced an immediate increase in coronary flow which lasted for 3-5 minutes. This was followed by a reduction in flow lasting for about 10 minutes. The increase in flow was associated with positive inotropic and chronotropic actions of the toxin on the heart; both these effects were blocked with dichloroisopropyl-noradrenaline (4 mg/kg, i.v.). The reduction in coronary flow, which followed, was associated with normal contractions of the heart. Administration of phenoxybenzamine at this stage produced an immediate increase in flow which became maximal in 7 minutes. Further doses of toxin, instead of producing any increase in flow, actually decreased it. The result of typical experiment has been shown in Fig. 3.

![Coronary Flow vs Time Graph](image)

**Fig. 3. Effects of tetanus toxin on coronary circulation of dog in vivo.**

*Injections of toxin and phenoxybenzamine given intravenously.*

Effects of tetanus toxin on hind limb perfusion of the rat

The effect of tetanus toxin on peripheral flow in perfused rat hind limbs was consistent in all the 8 experiments. The toxin produced 15-20% reduction in flow for 5-10 minutes. In animals pretreated with cocaine, the reduction was more marked (approximately 50%) and lasted for 20-30 minutes. Administration of reserpine to the animals, 24 hours before the experiment, abolished the effects of the toxin. Perfusion of phenoxybenzamine 30 minutes before the administration of the toxin had a similar effect (Fig. 4).
Fig. 4. Effect of tetanus toxin on blood vessels of hind limbs of rats perfused with oxygenated Ringer Locke solution at 37°C. Note the potentiation of effects after cocaine and the absence of effect in animals pretreated with reserpine or phenoxybenzamine.

Each arrow - Tetanus toxin - 1 ml
\[ \equiv 20,000 \text{ MLD/mice} \]

Fig. 5. Effect of tetanus toxin on peripheral blood flow in hind limb of dog. Injections of tetanus toxin given intraarterially.
Effects of tetanus toxin on peripheral blood flow of the dog

After the basal flow had stabilized, the toxin was injected intra-arterially. After a latent period of approximately 1/2 to 1 minute, there was a sudden and persistent reduction of 60-75% in the flow. Doses of phenoxybenzamine produced some increase in the flow. A second dose of the toxin, however, produced a further reduction in flow (Fig. 5).

Effects of tetanus toxin on blood sugar level

In fasting dogs, the toxin produced approximately 25%, increase in blood sugar levels, which was maximal in 15 minutes and lasted for about one hour. Injection of dichloroisopropyl-noradrenaline produced 30-40% reduction in blood sugar levels. Injections of toxin in such animals again produced a rise in blood sugar levels.

DISCUSSION

The resemblance of the actions of tetanus toxin with those of sympathomimetic amines was confirmed. Like the sympathomimetic amines, tetanus toxin produced an increase in rate and force of contraction of the heart, an increase in coronary flow, a reduction in peripheral blood flow and an increase in blood sugar levels. The delayed pressor effect was blocked by phenoxybenzamine, and the positive inotropic and chronotropic effects on the heart were blocked by dichloroisopropyl-noradrenaline. The initial increase in coronary flow was blocked by dichloroisopropyl-noradrenaline or phenoxybenzamine, but the later reduction was unaffected. The later reduction in coronary flow may, therefore, be presumed to be a direct effect.

The reduction in the coronary flow may be due to lowering of the blood pressure, oedema of the endothelial cells, or obstruction due to thrombus formation or embolisation and lastly it may be due to spasm of the coronary arteries. The decrease in the coronary flow occurred at a time when the blood pressure was actually elevated. A reversible decrease in coronary flow in the isolated perfused heart had been noted by us previously. In this set up, the reversibility of the change excludes obstruction either due to clots or due to endothelial oedema. The actual reduction is more likely to be due to vasospasm.

In the peripheral hind limb of the rat the action of the toxin was reversible. This was potentiated by cocaine and was absent in animals pretreated with reserpine or phenoxybenzamine. It is thus possible that some of the actions of the toxin may possibly be due to release of catecholamines.

The toxin produced a marked inhibition of flow in the hind limbs of the dog, and this was not affected by phenoxybenzamine and as such it is not likely to be due to sympathetic stimulation. Further, there was no measurable decrease in the catecholamine contents of adrenal gland, after systemic administration of the toxin.

The effect of tetanus toxin on the blood sugar levels was also not blocked by the adrenergic blocking agents. A severe reduction in the peripheral blood flow has been consistently seen to be produced by tetanus toxin. A reduction in blood flow through muscles leads to accumulation of toxic products and produces painful spasms. Thus one of
the reasons for painful tetanic spasms may be a reduction in blood flow through the muscles. The reduction in blood flow may be aggravated by muscle spasms, thus setting a vicious circle.

REFERENCES

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