

ADRENERGIC BLOCKADE PRODUCED BY THEPHORIN AND TOLAZOLINE HYDROCHLORIDE WITH SPECIAL REFERENCE TO THE BLOCKADE OF INTESTINAL RECEPTORS

By

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Thephorin and tolazoline hydrochloride both blocked vasopressor response of adrenaline, though tolazoline did it more intensely. Thephorin and tolazoline both blocked the relaxation of intestine induced by adrenaline *in vivo*. However both of these in smaller doses potentiated adrenaline responses on the intestine. Thephorin and tolazoline blocked the relaxation of isolated rabbit intestine induced by adrenaline. The block was reversible in both cases. But thephorin block was more intense and lasted for a longer time than the one caused by tolazoline hydrochloride. Thephorin and tolazoline blocked contractions of seminal vesicles induced by adrenaline, but the block was more intense and prolonged with thephorin compared to the one caused by tolazoline hydrochloride.

While studying the action of thephorin, an antihistamine drug on the vasodepressor response of adrenaline, Rajapurkar, Sachdev and Panjwani (1963), noted that pretreatment of the animal with thephorin caused a block of vasopressor response induced by adrenaline. In a higher dose range it produced a more pronounced block of vasopressor response of adrenaline and even vasodepressor response appeared. This property was in common with other adrenergic blocking agents. So the compound was studied further for its effects on adrenaline response on dog blood pressure.

It was found that extremely small concentrations of thephorin, blocked the responses to adrenaline on rabbit ileum. Since many adrenergic blocking agents are known to be incapable of effectively blocking the adrenaline induced relaxation of the intestine it was considered useful to study the adrenergic blocking activity of thephorin on intestine *in vitro* and *in vivo* and to compare it with the known adrenergic blocking agent priscoline. Intestinal relaxation is considered to be due to excitation of both *alpha* and *beta* receptors (Ahlquist and Levy, 1959). A study of the action of thephorin on the isolated guineapig seminal vesicle was carried out as it has principally *alpha* receptors.

METHODS

Twenty dogs of either sex weighing from 6 to 22 kg were used. Phenobarbitone sodium 150 mg/kg was given by intraperitoneal injection. Respiration was recorded by a tambour connected to a intratracheal cannula. Femoral vein was cannulated for intravenous injections of drugs. Carotid blood pressure was recorded by a mercury manometer.

Intestinal intraluminal pressure changes were recorded by means of a rubber balloon, which was manoeuvred into the lumen of intestine and fixed. The abdomen was closed but not made air tight purposely, so that the pressure changes in the abdominal cavity due to respiratory movements were not transmitted to the recording tambour. In order to obtain appreciable amplitude of the movements of intestine, magnification was increased by using a long writing arm.

Adrenaline hydrochloride was injected intravenously in graded doses varying from 0.5 $\mu\text{g}/\text{kg}$ to 3 $\mu\text{g}/\text{kg}$. Thephorin was given intravenously in a dose range varying from 1 mg/kg to 10 mg/kg at the rate of 1-2 ml/min. Tolazoline hydrochloride was administered intravenously in the dose range of 1.0 mg/kg to 10 mg/kg.

Ileum was excised from rabbit starved overnight and killed by a blow on the head. The loop was cleaned. This was suspended in a 20 ml. bath containing mammalian Ringer solution at 35-36°C, and continually bubbled with oxygen. Frontal writing lever was used for recording the movements. Adrenaline 0.012 $\mu\text{g}/\text{ml}$ to 0.5 $\mu\text{g}/\text{ml}$ was added to the bath for one min, and similar responses were elicited every three min. Thephorin 0.5 $\mu\text{g}/\text{ml}$ to 10 $\mu\text{g}/\text{ml}$ was added in the bath; three min. later the control doses of adrenaline were repeated. Priscoline was similarly tried in a dose range of 1 $\mu\text{g}/\text{ml}$ to 10 $\mu\text{g}/\text{ml}$. The time of recovery was noted.

Adult male guineapigs were killed by a blow on the head. The seminal vesicles were excised. It was suspended in mammalian Ringer solution at 35°-36°C, in a 20 ml bath. Atmospheric air was bubbled through Ringer's solution. Responses to adrenaline were elicited for 1½ min, every 5 min in a dose range of 0.5 $\mu\text{g}/\text{ml}$ to 2 $\mu\text{g}/\text{ml}$. Thephorin or priscolin was kept in the bath for three min and thereafter, adrenaline responses were elicited. Dose range tried were 0.5 $\mu\text{g}/\text{ml}$ to 5 $\mu\text{g}/\text{ml}$ of thephorin and 0.5 $\mu\text{g}/\text{ml}$ to 5 $\mu\text{g}/\text{ml}$ of tolazoline hydrochloride (Priscol CIBA).

RESULTS

Dog's blood pressure.—The action of thephorin on dog's blood pressure in dose range varying from 1-10 mg/kg given intravenously was studied in eleven dogs. Thephorin was injected after eliciting three control responses to adrenaline, varying between 0.5-3 μ g/kg. Thephorin was perfused slowly over 5-10 min. Five min. later, responses to control doses of adrenaline were obtained and compared for the degree of blockade.

Vasopressor responses caused by adrenaline were markedly reduced after thephorin (Fig. 1). In nine experiments out of eleven, adrenaline responses

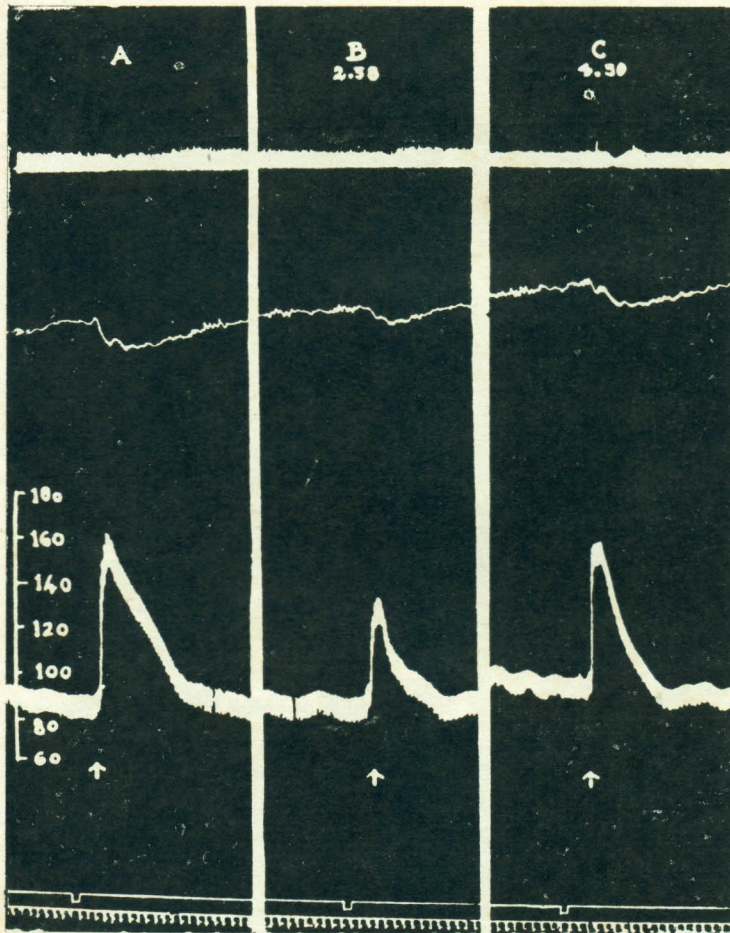


Fig. 1. Anaesthetised dog; record of respiration, intraluminal pressure changes of intestine, blood pressure and time tracings, from above downwards. At each arrow 1 μ g/kg of adrenaline was injected. Between Panel A and B thephorin 1 mg/kg was injected. Note a marked reduction in vasopressor response and intestinal relaxation in Panel B, partial recovery is seen in Panel C.

after thephorin perfusion were biphasic. An initial pressor response was followed by a weak depressor response. In one experiment the pressor response was small and depressor response was marked. In none of the experiments the initial vasopressor response was completely abolished. Adrenergic block persisted for 2-3 hrs. Maximal blockade developed within half an hour of administration of thephorin. After about an hour the block became progressively weaker and the vasopressor response gradually increased. Full recovery was not obtained in any of the experiments for $2\frac{1}{2}$ hrs. The adrenergic block varied from 8-73 per cent of the original response. When the log dose of thephorin was plotted against percentage block a fairly good straight line was obtained.

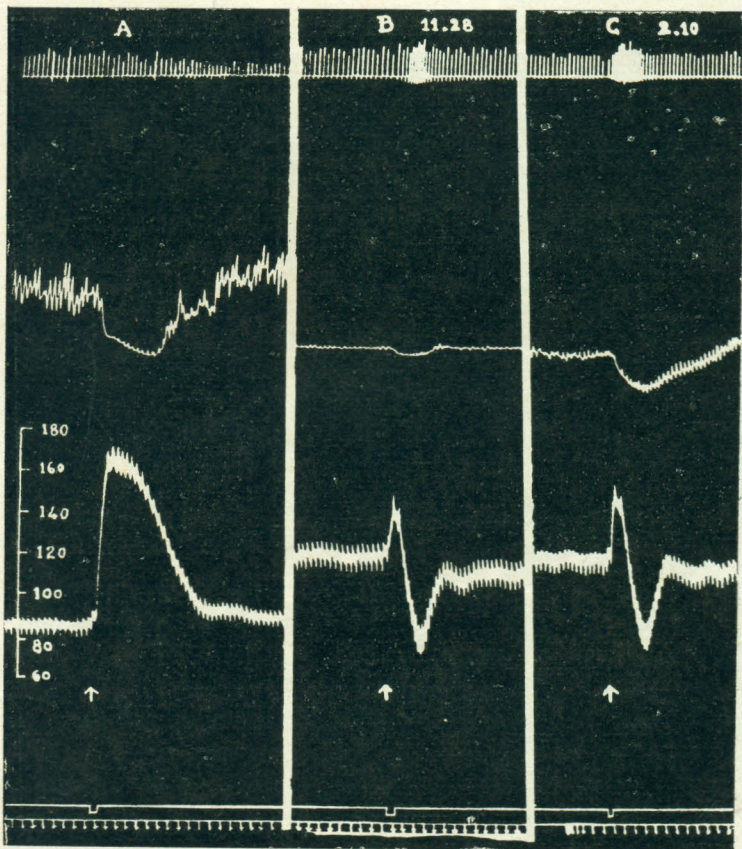


Fig. 2. Anaesthetized dog; record of respiration, intraluminal pressure changes of intestine, blood pressure and time tracings, from above downwards. At each arrow $1 \mu\text{g}/\text{kg}$ of adrenaline was injected. Between Panel A and B tolazoline $5 \text{ mg}/\text{kg}$ was injected. Note that tolazoline causes blockade of adrenaline vasopressor response and intestinal relaxation; Panel B, Panel C. show a differential recovery.

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Tolazoline in doses varying between 1.0 mg/kg to 10 mg/kg modified adrenaline vasopressor response markedly. Vasopressor response was blocked in proportion to the dose of prisoline used. As the dose of prisoline was increased the vasopressor response became smaller and the vaso depressor response more intense Fig. 2. Higher dose range of tolazoline abolished initial pressor response i.e. only depressor response was obtained with adrenaline. The peak effect developed in about half an hour. The block steadily persisted for more than two hrs. Tolazoline caused a greater block which lasted for a longer time compared to that produced by the same dose of thephorin. Thephorin block faded earlier than prisol block. Reversal with thephorin was not pronounced.

Thephorin and tolazoline both caused tachycardia at the time of administration. Thephorin did not change blood pressure level, but tolazoline caused a rise of blood pressure. Neither of the drugs caused cardiac arrhythmias or respiratory depression.

Dog's intestine.—0.5 μ g/kg to 2 μ g/kg of adrenaline given intravenously caused relaxation of the dog's intestine. The extent of block caused by 1 mg/kg to 10 mg/kg of thephorin varied from a small diminution of the relaxation to complete absence of relaxation Fig. 1. In three experiments complete recovery was obtained. The block developed within 5-10 min after thephorin, and decreased in about 1½ hrs. Recovery of intestinal block was quicker than that of the blood pressure responses.

Very small doses of thephorin 0.5 mg/kg caused a paradoxical augmentation of the intestinal relaxation produced by adrenaline. Recovery to original level was obtainable in about 15-20 min.

Tolazoline was used in dose range of 1.5 mg/kg intravenously. It caused a marked rise in tone of intestine and increased the spontaneous activity. The relaxation of intestine caused by various doses of adrenaline ranging from 0.5 to 2 μ g/kg was potentiated by 1 mg/kg of tolazoline. But higher doses blocked the relaxation considerably, Fig. 2.

Isolated intestine of rabbit :—Thephorin 0.5 μ g/ml to 10 μ g/ml blocked adrenaline responses, Fig. 3a. The block was more marked and total with higher dose of thephorin, Fig. 5. The block persisted for 15 to 45 min. Full recovery was obtained in thirteen out of twenty-four assays. In the remaining ones recovery was partial.

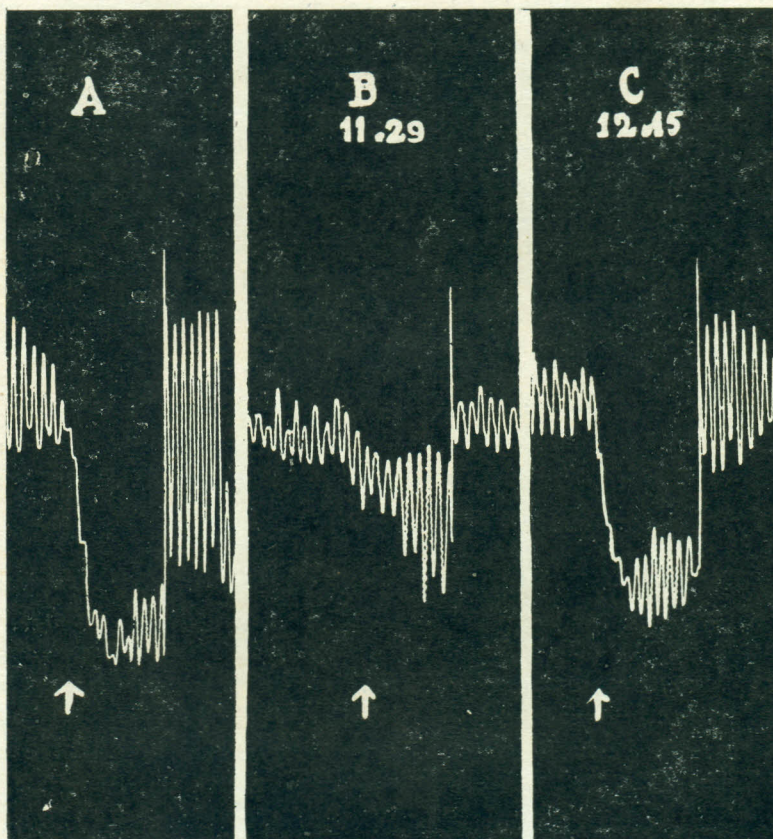


Fig. 3 a. Isolated intestine of rabbit. At each arrow $0.04 \mu\text{g/ml}$ of adrenaline is added to the bath. $1.00 \mu\text{g/ml}$ of thephorin was added to the bath 3 min before the response in Panel B. Thephorin has blocked adrenaline induced relaxation.

Tolazoline hydrochloride $5 \mu\text{g/ml}$ blocked adrenaline induced relaxation, Fig. 3b. The block was proportional to the amount of tolazoline in the range of $0.5 \mu\text{g/ml}$ to $10 \mu\text{g/ml}$.

Recovery was complete within 5-10 min. 100 per cent block of relaxation was not obtained in any experiment. Full recovery was obtained in all the fourteen assays. Neither of the drugs changed the tone, amplitude and rhythm of spontaneous intestinal movements. Thephorin caused more prolonged and intense block than tolazoline.

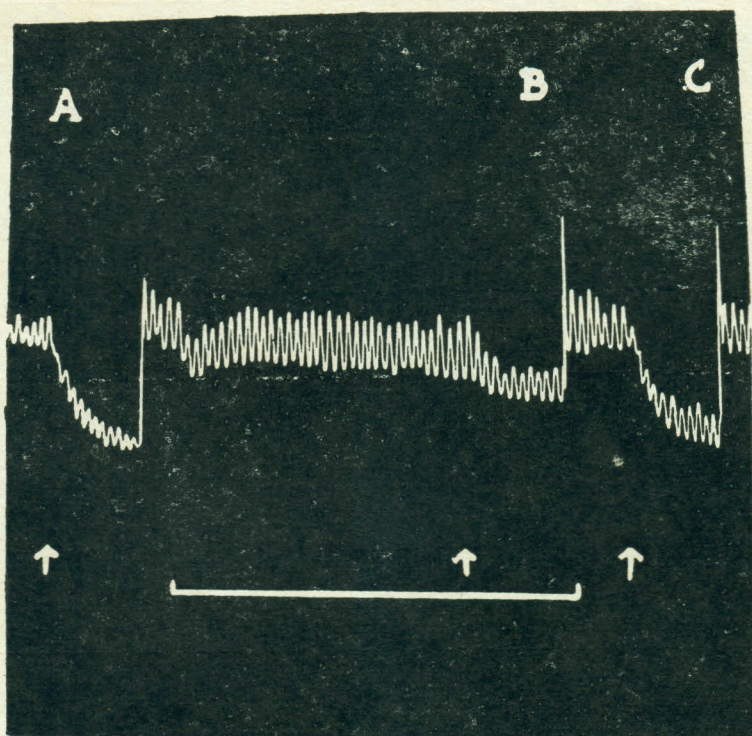


Fig. 3 b. Isolated intestine of rabbit. At each arrow 0.05 $\mu\text{g/ml}$ adrenaline hydrochloride was added to the bath. Tolazoline hydrochloride 5.0 $\mu\text{g/ml}$ was injected at the bar; note that 5 times the dose of theophorin in Fig. 3 a. caused a partial block and recovery was very rapid.

Guineapig seminal vesicle:—Adrenaline induced contractions were blocked by theophorin in the dose range of 0.5 $\mu\text{g/ml}$ to 10 $\mu\text{g/ml}$ Fig. 4. Higher doses of theophorin caused a greater block of adrenaline induced contractions Fig. 6. The duration of block was usually 25-35 min.

Tolazoline 1.0 $\mu\text{g/ml}$ to 5 $\mu\text{g/ml}$ caused a block of adrenaline response Fig. 7. The block faded in a short time. Recovery was complete in all the trials, within 10-15 min. Theophorin caused a more pronounced and prolonged block than tolazoline Fig. 7.

DISCUSSION

Thephorin, an antihistaminic drug, blocked and some times reversed the pressor response of adrenaline on dog blood pressure. It blocked the

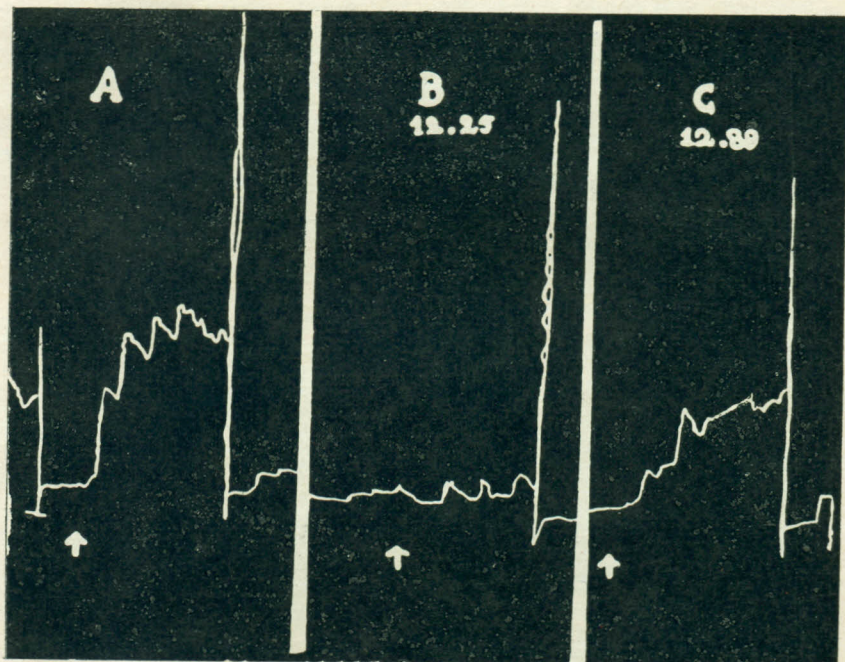


Fig. 4. Guineapig seminal vesicles *in vitro*. At each arrow $0.25 \mu\text{g} / \text{ml}$ of adrenaline was added to bath. Between A and B theophorin $3.0 \mu\text{g} / \text{ml}$ was injected. Response of Panel B was taken 3 min after addition of theophorin. Note that the adrenaline response is markedly blocked after theophorin (Panel B). Panel C shows partial recovery at 12:39.

adrenaline induced relaxation of dog intestine *in vivo* and rabbit intestine *in vitro*. Theophorin also blocked contractions of isolated guineapig seminal vesicle induced by adrenaline. However, in smaller dose range it potentiated the relaxation of intestine produced by adrenaline both *in vitro* and *in vivo*.

Tolazoline hydrochloride in doses of $1.0 \text{ mg} / \text{kg}$ to $10 \text{ mg} / \text{kg}$ blocked adrenaline pressor responses, and reversed these.

Compared to tolazoline, theophorin in the same dose range caused similar but less intense block of the vasopressor response of adrenaline.

The adrenergic blocking activity of theophorin is probably due to α receptor blockade. Vasodepressor response to adrenaline after theophorin was infrequent compared to tolazoline. In this way it differs from other α receptor blocking agents. Absence of strong vasodepressor response with

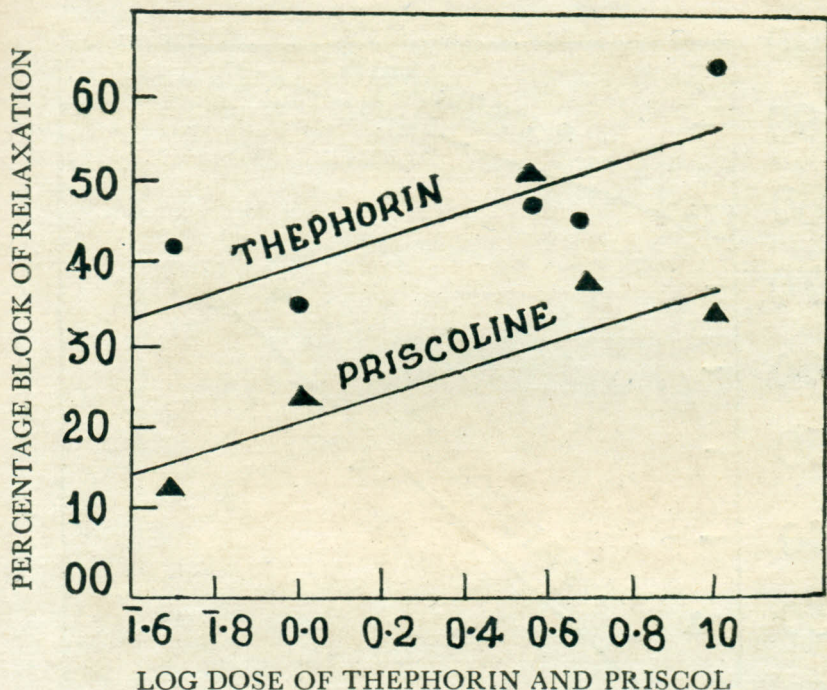


Fig. 5. The action of adrenaline by thephorin and tolazoline on isolated rabbit intestine *in vitro*. Thephorin causes more intense block than tolazoline *in* all dose ranges.

adrenaline after thephorin may be due to an associated *beta* receptor blocking activity. The potent blockade of the depressor response of adrenaline on isolated intestine, supports this possibility.

The mechanism of action of thephorin as an adrenergic blocking agent is not clear. There is no apparent chemical similarity between the usual adrenergic blocking agent and thephorin.

Björbring (1960) has put forward a general hypothesis about the excitatory and inhibitory responses of adrenaline. She has postulated that changes in the permeability of the cell membrane, or its stabilisation by metabolic activity of adrenaline, ultimately decides tissue response either in form of contraction or relaxation. This according to her is an alternative to Ahlquist's receptor theories. As an antihistaminic, thephorin might be affecting permeability of the cell membrane resulting in inhibition of vasopressor response of adrenaline. But the other antihistaminics not only do not block the action of adrenaline but enhance it, Innes (1958).

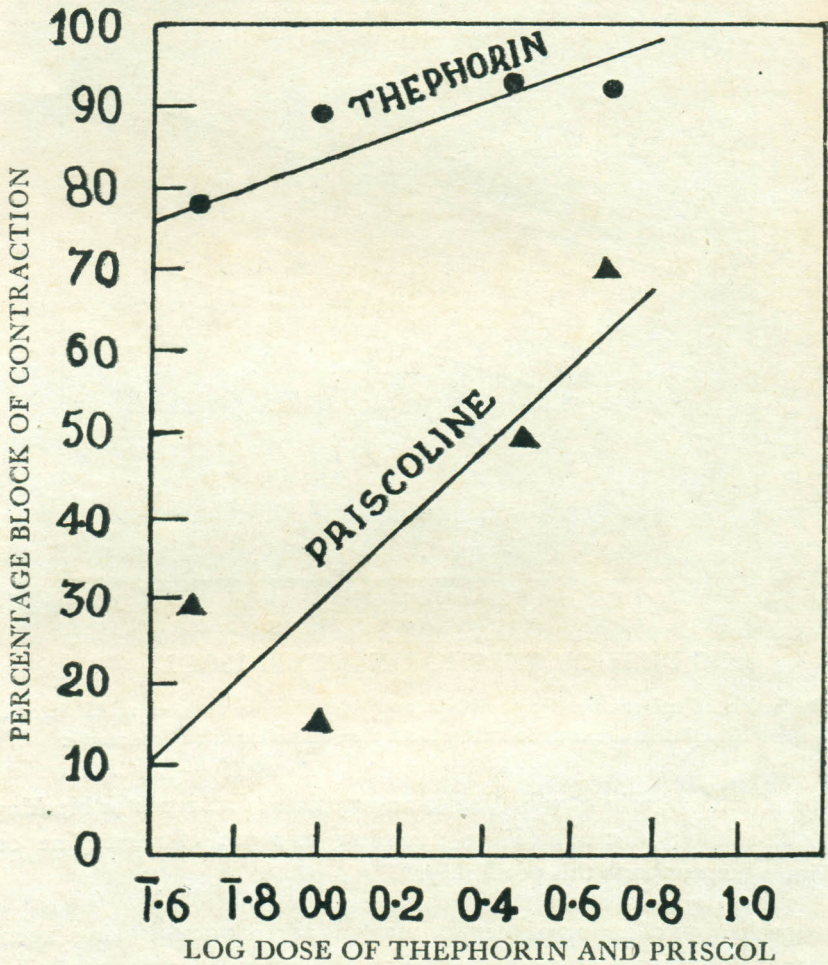


Fig. 6. Blocking action of adrenaline by thephorin and tolazoline on isolated guineapig seminal vesicle, showing percentage block of contraction. Thephorin causes a greater block than tolazoline in all dose ranges.

In the present work tolazoline has been shown to antagonise relaxation of intestine caused by adrenaline, *in vitro* and *in vivo*. Veragic (1956) showed that tolazoline blocked the response of the isolated rabbit colon to the stimulation of lumbar colonic nerve.

There are conflicting reports about the action of, adrenergic blocking agents on the intestinal responses to sympathomimetic amines. Goodman and Gilman (1955) state that the predominant effects of adrenergic stimuli on

the gastro-intestinal tract are inhibitory, since the *beta* haloalkylamines fail to block inhibitory sympathetic responses, they have only a limited action on enteric tract. The relaxation of isolated intestinal muscle caused by epinephrine and other sympathomimetic drugs is not significantly altered by usually effective doses of dibenamine and its congeners.

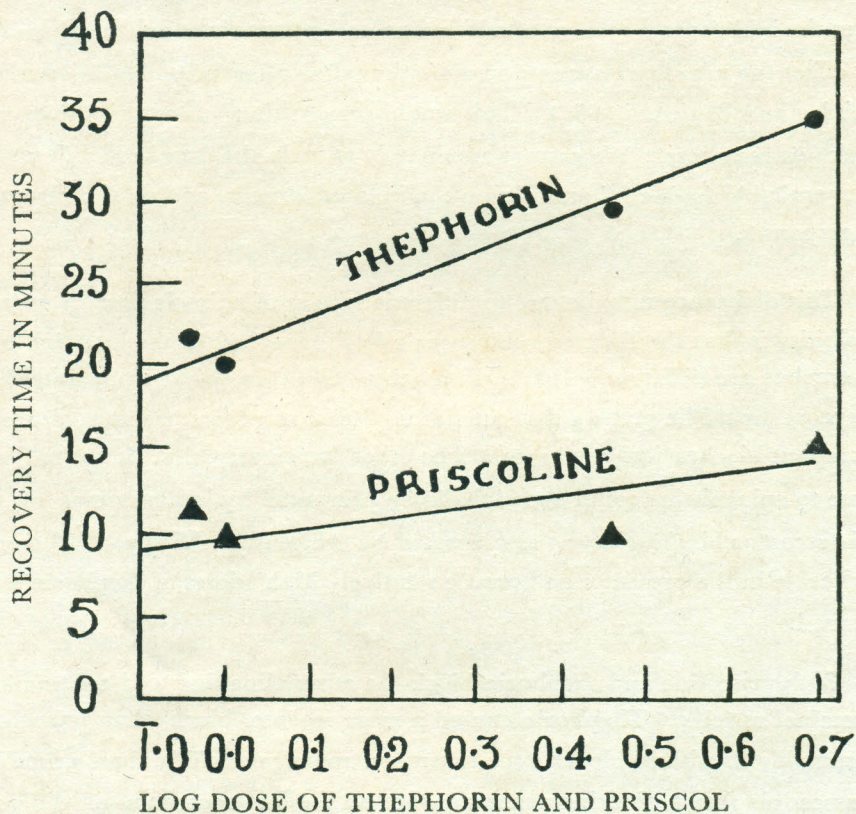


Fig. 7. Blockade of action of adrenaline by thephorin and tolazoline on isolated guineapig seminal vesicle, showing recovery time. Thephorin took longer time for recovery than tolazoline hydrochloride in any dose range tried.

Drill (1958) mentions that the inhibitory action of epinephrine on the intestine *in vitro* is blocked by high doses of dibenamine.

Ahlquist and Levy (1959) found that to antagonise effectively the effects of adrenaline, which act on both types of receptors one would need the simultaneous presence of antagonists of both *alpha* and *beta* receptors. Since small doses of thephorin alone effectively block intestinal relaxation caused by adrenaline both *in vivo* and *in vitro* it is possible that thephorin blocks both *alpha* and *beta* receptors.

Compared to tolazoline, thephorin causes a weaker block of the vasopressor response of adrenaline, and yet its greater block of the adrenaline induced contraction of seminal vesicle and relaxation of intestine may be due to its combined *alpha* and *beta* receptors blocking action. However, thephorin potentiates the inhibitory responses of adrenaline on guineapig trachea and does not effect the vasodepressor response of adrenaline after priscol (Rajapurkar, Sachdev and Panjwani 1963). This would suggest that thephorin does not block *beta* receptors. We cannot adequately explain the marked differential adrenergic blockade of thephorin on the isolated organs, and on the vasopressor responses.

This difference may be due to different species of animals used. A greater possibility is that the depressor adrenergic *beta* receptors in the intestine and the trachea are different. Infact, Furchgott (1960) proposed that intestinal receptors should be given a distinct status of *delta* receptors because of many discrepant biochemical responses. The other possibility that intestinal response to epinephrine could occasionally be prevented by larger doses of the *alpha* receptor blocking agent as suggested by Ahlquist and Levy (1959) is not applicable in the present case because relatively high doses of thephorin were not used.

Both priscoline and thephorin block the adrenaline induced contractions of seminal vesicles. Thephorin caused a more intense block than priscoline. Presumably thephorin blocks *alpha* receptors, more powerfully than priscoline. It is possible that a blocking agent may act on similar receptors of different organs with different intensity. From the present work it can not be said whether thephorin produces a competitive or noncompetitive block. But the action of thephorin is reversible and yet very persistent.

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