THE EFFECTS OF THIAMINE TETRAHYDROFURYL DISULFIDE UPON THE MOVEMENT OF THE ISOLATED SMALL INTESTINE

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Recently Hukuhara, Nanba and Siina (3) established that thiamine tetrahydrofuryl disulfide (TTFD) had excitatory effect upon the motility of the normal and decentralized Thiry-Vella loops when the drug was administered intravenously or applied to the mucosa, whereas it had no effect upon the motility of the loop, whose intramural nervous elements had been completely destroyed. From these results they presumed that the drug could exert an excitatory action upon the intramural nervous elements directly as well as reflexly, whereas it had no action upon the intestinal muscle. In order to obtain further informations about the site of the action of the drug a series of experiments were carried out on the isolated small intestine of various mammals.

EXPERIMENTAL

Methods

As experimental animals in most cases guinea pigs and dogs and sometimes rabbits and cats were used. In guinea pigs the abdominal cavity of the non-anesthetized animal was opened to remove the ileal, jejunal or duodenal loop of about 30 mm in length. Utilizing Trendelenburg’s method (12) the change of length as well as of volume of the loop was recorded on the kymograph under the isotonic condition. The constituents of the Tyrode solution were modified after Takemasa (10, 11).

In dogs and cats a duodenal or jejunal piece, 20 mm in length, was removed from the animal anesthetized by intravenous administration of 25 mg/kg pentobarbital sodium. After the piece was incised along its mesenteric border and immersed into cooled Tyrode solution kept at about 4°, the submucosa together with the mucosa was stripped off the piece. Two kinds of preparations, ‘ganglion cell containing’ and ‘cell free’, were obtained from this piece. The former preparation was obtained by cutting the piece into strips, about 20 mm long and 1.5—2.0 mm wide, along the direction of the circular muscle. The latter preparation was a bundle of circular muscles stripped off the piece, being measured about 20 mm

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long and 1.5—2.0 mm wide. Both kinds of preparations were also prepared from the intestine cooled at 1.5° for 20—24 hours. It must be noted here that the terms 'cell containing' and 'cell free' are erroneous but for convenience sake the present authors retain the term 'cell' to denote the cell body. In rabbits were used the strips which contained all the intestinal layers.

The contractions of strip preparations were recorded on the kymograph after Magnus' method (6). After the termination of the experiments the preparations were stained supravitaly with methylene blue to determine whether they contained ganglion cells or not, staining solution being consisted of 8.0 g NaCl, 0.3 g resorcinol, 0.3 g methylene blue and 1,000 ml distilled water (9).

The drugs used were TTFD (Takeda), physostigmine sulfate (Merck), acetylcholine chloride (Daiichi Seiyaku), nicotine bitartrate (Katayama), Hexamethonium bromide (Ciba) (Yamanouchi), morphine hydrochloride (Takeda), atropine sulfate (Merck) and reserpine (Ciba). They were dissolved in distilled water, care being taken not to add more than one ml of these solutions to avoid the effect of dilution of the Tyrode solution on the preparations.

RESULTS

1. Influence of TTFD upon the Motility of Isolated Guinea-Pig Small Intestine

When TTFD was added in concentration of $5 \times 10^{-5}$ mg/ml in the bath, in which guinea-pig intestinal loop was immersed, and its motility was recorded by means of Trendelenburg's method. Such effects were observed that the excitatory effect was intimately associated with the inhibitory one: The volume curve, illustrated in Fig. 1, showed that the tone was slightly lowered, and the time interval of individual contractions was slightly decreased, whereas the amplitude of contractions was remarkably increased.

![Fig. 1](effect_of_ttfd upon_motility_of_isolated_guinea-pig_small_intestine.png)

**Fig. 1** Effect of TTFD upon the Motility of the Isolated Guinea-Pig Jejunal Loop

Trendelenburg's method. Tracings from above downwards, change in the longitudinal direction as well as in volume of the loop, signal indicating the moment of application of the drug and time in seconds. Explanations in the text.
That the behavior of individual contractions could not always be relevantly judged from the changes occurred in the longitudinal direction of the loop had been emphasized by Hukuhara and Yokoyama (4), and Hukuhara and Fukuda (5), so that the length curve was illustrated for only a reference sake.

Such effects as described above were definitely produced, when the intestinal tone was raised at a high level by previous application of morphine, the excitatory effect of which had been studied in our unpublished experiments. The example is shown in Fig. 2. When TTFD was added in the concentration of $5 \times 10^{-5}$ g/ml in the bath, the tone was lowered, and individual contraction waves were prolonged in their period but remarkably increased in their amplitude.

2. Experiments Concerning the Site of Action of TTFD

Since TTFD exerted no action upon the motility of the intestine whose intramural nervous elements had been completely destroyed, the drug is supposed not to act upon the intestinal muscle itself (3). The corollary is that the drug may act upon the nervous elements. From the results obtained in the experiments concerned with the intestinal intrinsic reflexes Hukuhara et al. (1, 2, 5), suggested that there existed two kinds of neurones in the intestinal wall. If this is true, so it would be presumed that the drug concerned acts upon the neurones to elicit the effects described above. The evidences were obtained in the following experiments.

As shown in Fig. 3 A, TTFD produced in the concentration of $5 \times 10^{-5}$ g/ml an excitatory effect upon the rabbit duodenal strip, the motility of which was recorded by means of Magnus’ method. However, after adding atropine in the concentration of $10^{-4}$ g/ml in the bath, the effect of TTFD, in the same concentration as before, reversed to an inhibitory one lasting for a considerably long time, as shown in Fig. 3 B. Nicotine is regarded as a ganglion stimulating agent, which sometimes excites and sometimes inhibits the intestinal motility. However, this drug in the concentration of $10^{-4}$ g/ml always produced an inhibitory effect on the intestinal motility,
Magnus’ method. At W the bath fluid was exchanged with the fresh one. TTFD added at Ai produced an excitatory effect (A), which was reversed to the inhibitory one after applying atropine (At) to the strip (B). Nicotine produced an inhibitory effect after atropine (C). No action of TTFD was observed after atropine and C6 (D).

When atropine had previously been added in the concentration of $10^{-4}$ g/ml in the bath, as shown in Fig. 3 C. On the basis of the results mentioned above the reversal of the effect of TTFD is supposed to be produced, because the function of excitatory neurones is suppressed by atropine, whereas that of inhibitory ones is left intact.

When the administration of atropine was followed by that of C6, concentration of the drugs being $10^{-4}$ g/ml and $5 \times 10^{-5}$ g/ml respectively, TTFD hardly had an effect upon the intestinal motility (Fig. 3 D). The ineffectiveness of TTFD may be due to the suppression of the function of respective neurones by atropine or C6, and on the other hand, this fact suggests that TTFD exerts no action upon the intestinal muscle.

In order to suppress the function of inhibitory neurones the cat was treated with reserpine: Reserpine was subcutaneously administered on the first day in the dose of 1.5 mg/kg and on the second day in 2.5 mg/kg. On the third day the outer muscle strip containing ganglion cells in Auerbach’s plexus was prepared from the duodenum of the animal. The strip showed an undulation of tone superimposed with rhythmic contractions, as illustrated in Fig. 4. When TTFD was added in the concentration of $5 \times 10^{-6}$ mg/ml, a remarkable rise of the intestinal tone was produced. This rise of tone may be explained by the predominance of the function of the excitatory neurone, that resulted from the exhaustion of the transmitter
TTFD added at Al produced an enormous rise of tone.

In addition, when on the cat previously treated with reserpine 0.7 mg/kg atropine was intravenously administered, TTFD was ineffective on the motility of the strip (Fig. 5). In this case it could be presumed that the mechanism for transmission of excitation not only from inhibitory but also excitatory neurones to the muscle would be abolished by the drugs applied. And this ineffectiveness suggests again that TTFD has no action on the intestinal muscle.

In ‘ganglion cell containing’ strips prepared from the intestine cooled at 1.5° for 20—24 hours the characteristic features of TTFD effect, that is, lowering of tone, prolongation of period and increase of amplitude were much more pronounced than in fresh preparations, and in ‘ganglion cell free’ strips only the inhibitory effect was marked. The example is shown in Fig. 6.

The saturated water solution of TTFD crystal produced the effects similar to those observed when the drug for injection was administered, and the solvent of the drug was ineffective on the ‘cell containing’ as well as ‘cell free’ strip, the example being shown in Fig. 7.
DISCUSSION

From the results obtained in the previous (3, 7, 8) and present experiments concerned with the action of TTFD upon the intestinal motility it may be concluded that the drug has an excitatory action on the intramural nervous elements but no effect on the intestinal muscle. Fig. 8 illustrates schematically the essential components which are concerned with the drug action. According to Hukuhara et al. (1, 2, 5) the excitatory and inhibitory neurones are supposed to exist in Auerbach's plexus, and synaptically to connect with the preganglionic parasympathetic nerve.
fiber. And these neurones play, being associated with neurones in Meissner's plexus, a role of center of the mucosal intrinsic reflex. The mechanism of action of TTFD may be explained as follows: Firstly, TTFD stimulates the intestinal mucosa to elicit the mucosal intrinsic reflex. Secondly, the drug can directly exert the excitatory action upon both neurones, resulting in the summated effects on the intestinal motility. And in vivo it may be only the excitatory action that can be recognized, because of the predominance of the function of the excitatory neurone over that of the inhibitory one, whereas in vitro the inhibitory action also manifests itself, being probably due to the changes of excitability of neurones. In addition, the nerve fiber, indicated with broken line in Fig. 8, is the sympathetic nerve fiber which is supposed to connect synaptically with the excitatory neurone. The evidence that this kind of fiber exists has been obtained in our unpublished experiments.

SUMMARY

Utilizing Trendelenburg's as well as Magnus' method, the influence of thiamine tetrahydrofurfuryl disulfide (TTFD) upon the movement of the isolated small intestine of guinea pigs, rabbits, cats and dogs was studied. The results were summarized as follows:

1. At the concentration of $5 \times 10^{-5}$ g/ml TTFD produced on the one hand a
lowering of the tone, prolongation of the period of rhythmic contraction waves and on the other hand a remarkable increase of the amplitude of the waves.

2. After the administration of atropine the excitatory effect of TTFD was reversed to the inhibitory.

3. After successive administrations of atropine and C₆ no action of TTFD was marked.

4. TTFD always had an excitatory effect upon the motility of the intestinal strip prepared from the animal which had been treated with reserpine.

5. TTFD had no effect on the small intestine isolated from cats treated with reserpine and atropine.

6. From the results described above it may be concluded that TTFD exerts an excitatory action upon both the excitatory and inhibitory neurones residing in Auerbach’s plexus, whereas it exerts no action upon the intestinal muscle. It could be considered that the effects described in (1) were the results of a mutual coordination of the function of two kinds of the neurones described above.

REFERENCES

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