POSSIBLE MECHANISMS OF A NEW TYPE OF ANTISpasMODIC DRUG, BTM-1042(CIS-(−)-2,3-DIHYDRO-3-(4-METHYL-PIPERAZINYL)METHYL-2-PHENYL-1,5-BENZOTHIAZEPIN-4(5H)-ONE DIHYDROCHLORIDE)

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Abstract—A newly synthesized compound, BTM-1042 (cis-(−)-2,3-dihydro-3-(4-methyl-piperazinyl)methyl-2-phenyl-1,5-benzothiazepin-4(5H)-one dihydrochloride) depressed the twitch responses of the ileum from guinea pig to electrical stimulation at 0.1 Hz. This inhibitory action of BTM-1042 was not influenced by naloxone, a narcotic antagonist. BTM-1042 proved to be almost as active as atropine on electrically stimulated ileum. BTM-1042 also blocked muscarinic receptors but the potency was about 1/13 of that of atropine. The responses of the ileum of guinea pig to nicotine and 5-hydroxytryptamine also were inhibited by BTM-1042. However, BTM-1042 did not influence the release of transmitters from motor, sympathetic, nonadrenergic inhibitory (or purinergic nerve), noncholinergic excitatory nerves and responses of various smooth muscles mediated through drug receptors, except for the acetylcholine receptor. Spontaneous movement of the unanaesthetized rabbit stomach was dose dependently depressed by BTM-1042 (0.04-0.2 mg/kg, i.v.). The potency ratio for BTM-1042 relative to atropine was 7.4. BTM-1042 is apparently a new type of potent, antispasmodic drug.

During investigations of the antispasmodic action of newly synthesized compounds, a new type of antispasmodic drug, BTM-1042 was found to have a marked suppressive effect on spontaneous movement of the stomach in conscious rabbits. Attempts were made to determine the sites of action of BTM-1042 on the gastrointestinal system and the mode of action of BTM-1042 on various muscle tissues.

MATERIALS AND METHODS

The test compound, BTM-1042 is a white powder which is soluble in water and the chemical formula is shown in Fig. 1. BTM-1042 was synthesized in the laboratory of Maruko-Seiyaku Co. Ltd. (Nishi-ku, Nagoya, Japan). Other drugs used herein were

![Fig. 1. Chemical formula of BTM-1042 (cis(−)-2,3-dihydro-3-(4-methylpiperazinyl)methyl-2-phenyl-1,5-benzothiazepin-4(5H)-one hydrochloride).]
acetylcholine chloride, 5-hydroxytryptamine creatinine sulfate, histamine dihydrochloride, nicotine bitartarate, epinephrine hydrochloride, isoprenaline hydrochloride, atropine sulfate, guanethidine sulfate, procaine hydrochloride, d-tubocurarine chloride and tetrodotoxin, all in powder form.

Isolated muscle preparations used in this study were suspended in a 20 ml organ bath filled with a physiological solution kept at 37°C and gassed with a mixture of 95% O₂ and 5% CO₂. The physiological solution used had the following composition: NaCl 0.9 g, CaCl₂ 0.2 g, MgCl₂ 0.1 g, NaHCO₃ 1 g and glucose 1 g in a litre.

Isolated ileum, tracheal muscle preparation and taenia caecum: Guinea pigs, weighing 300 to 400 g were sacrificed by a blow on the head and ileum, trachea and taenia caecum were isolated and placed in physiological solution. The tracheal muscle preparation was made by the method of Takagi and Takayanagi (1). Pieces (3 to 4 cm) of the ileum and taenia caecum were also used. Responses to drugs were recorded isotonically under a tension of 0.5 g. In some experiments, the ileum was stimulated electrically by the method of Paton (2). The electrodes were made of platinum and the intraluminal electrode was used as the anode. Rectangular pulses of 0.1 msec duration were used at a frequency of 0.1 Hz and at a voltage sufficient to give a maximal response. The perivascular nerve-causeum preparation was prepared by the method of Burnstock et al. (3). To stimulate the nonadrenergic inhibitory nerve (purinergic nerve), the taenia caecum was placed between two platinum electrodes and stimulated with rectangular pulses of 0.1 msec duration and supramaximal voltage at 10 Hz for 2 sec (3). Responses of the taenia caecum and ileum to electrical stimulation were recorded isometrically with an initial tension of 4.0 g.

Fundus strip preparation: The stomach was removed from rats weighing 200 to 300 g and strip preparations were made using the method of Vane (4). Responses to drugs were recorded isotonically under a tension of 0.5 g as mentioned above.

Isolated urinary bladder: The urinary bladder of guinea pig was used, as in this organ, the adrenergic innervation is weak, if present. Guinea pigs weighing 300 to 450 g were sacrificed and the urinary bladder (5) was placed in physiological solution kept at 37°C and gassed with the mixture of 95% O₂ and 5% CO₂. Field stimulation of the bladder was carried out by passing a rectangular pulse of 1 msec duration, supramaximal voltage and a frequency of 0.1 Hz between two platinum electrodes. The preparation responded to a single pulse. The twitch responses to electrical stimulation were recorded isometrically with an initial tension of 0.1 g.

Isolated vas deferens and phrenic nerve-diaphragm preparation: The vas deferens was carefully removed from male rats weighing 200 to 250 g. Field stimulation of the vas deferens was also achieved by passing a rectangular pulse as mentioned in the experiments with the urinary bladder. The preparation responded to a single pulse and the twitch responses to electrical stimulation were recorded isometrically with the initial tension of 1.0 g. The diaphragms with phrenic nerve were removed from male rats (200 to 300 g in body weight) and preparations made by the method of Bülbring (6) were suspended in 50 ml organ bath filled with the physiological solution and stimulated indirectly via Ag-AgCl
electrodes. Pulses applied to the phrenic nerve were of 1 msec duration at 10 Hz for 1 sec at intervals of 30 sec. The twitch responses to electrical stimulation were also recorded isotonically with an initial tension of 0.1 g.

Spontaneous movement of the unanaesthetized rabbit stomach: Male rabbits weighing 2 to 2.5 kg were anaesthetized with sodium pentobarbitone (35 mg/kg, i.v.) and a rubber microballoon was implanted into the smooth muscle layer of the pyloric antrum. At least three days after the implantation and after being fasted for 24 hr, the conscious rabbits were used. The initial pressure of the balloon was adjusted to 5 cm H2O during the smooth muscle relaxation. Changes in the internal pressure of the balloon were recorded by a low pressure transducer. Normal saline solution was as used as a vehicle and all doses of the drug were given in 1 ml/kg i.v. into the auricular vein (7).

RESULTS

To determine the interaction between BTM-1042 and agonists and antagonists, the muscle preparations were preincubated with BTM-1042 or one of the antagonists for 5 min. BTM-1042 (3 × 10⁻⁸ to 3 × 10⁻⁷ g/ml) induced a parallel shift of the dose response curve of acetylcholine, thereby indicating a competitive antagonism (Fig. 2). Negative logarithm of the dose (g/ml) necessary to reduce the effect of a double dose of the agonist to that of the single dose was expressed as a pA₂ value, as calculated from the table of van Rossum (8). The pA₂ value of BTM-1042 was 8.26±0.13 (mean±S.E. of 8 experiments), while that of atropine was 9.43±0.08. Atropine-like activity of BTM-1042 was about 1/13 of atropine. Response to histamine was slightly depressed by BTM-1042 (10⁻⁵ g/ml). BTM-1042 (up to

![Graph showing contraction of guinea pig ileum](image)

**Fig. 2.** Effects of BTM-1042 on dose response curves of acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) tested on the isolated guinea pig ileum. From the left to right: control curve, with BTM-1042 (3 × 10⁻⁸ g/ml), with BTM-1042 (10⁻⁷ g/ml) with BTM-1042 (3 × 10⁻⁷ g/ml). Values are presented as means of 8 experiments.
$10^{-5} \text{ g/ml}$ did not influence the dose response curves of 5-hydroxytryptamine in rat fundus strips, epinephrine in the rat vas deferens and isoprenaline in the guinea pig tracheal preparation. Five min treatment of the guinea pig ileum with BTM-1042 resulted in a shift of the dose response curves of 5-hydroxytryptamine (Fig. 2) and of nicotine with a decline of slope. The equipotent dose of nicotine and 5-hydroxytryptamine was, respectively, $3.0 \times 10^{-6} \text{ g/ml}$ and $1.0 \times 10^{-6} \text{ g/ml}$. The dose of BTM-1042 required to inhibit the response to nicotine, $3.0 \times 10^{-6} \text{ g/ml}$ to 50% was $(5.3 \pm 0.3) \times 10^{-8} \text{ g/ml}$, this being much the same as the dose of BTM-1042, $(3.8 \pm 0.3) \times 10^{-8} \text{ g/ml}$ necessary to inhibit the response to 5-hydroxytryptamine, $1.0 \times 10^{-6} \text{ g/ml}$ to 50%. These values are presented as the means ± S.E. of 8 experiments.

BTM-1042 ($10^{-8}$ to $10^{-7} \text{ g/ml}$) dose dependently inhibited the twitch response of the ileum to electrical stimulation at 0.1 Hz and was almost as active as atropine and about 100 times as active as procaine (Fig. 3), used as a reference drug for local anaesthetic action. This inhibitory effect of BTM-1042 was not influenced by naloxone $10^{-7} \text{ g/ml}$, a dose which would antagonise the effect of morphine (Fig. 4).

Responses of the taenia caecum to perivascular nerve stimulation and transmural stimulation at 10 Hz were not influenced by a 10 min treatment with BTM-1042 ($10^{-8} \text{ g/ml}$). In addition, responses to transmural stimulation were not influenced by BTM-1042 (up to $10^{-6} \text{ g/ml}$) in the presence of guanethidine ($10^{-6} \text{ g/ml}$), indicating that BTM-1042 did not influence the inhibitory response to nonadrenergic nerve (purinergic nerve) stimulation (Fig. 5).

It is well known that the response of the urinary bladder to nerve stimulation is not abolished by a muscarinic receptor blockade. In our study, the urinary bladder continued

![Inhibition(%) Guinea pig ileum](image)

**Fig. 3.** Effects of BTM-1042, atropine and procaine on the twitch response of guinea pig ileum to electrical stimulation. Values are presented as means ± S.E. of 6 experiments.
to respond to transmural stimulation in the presence of atropine (3 × 10⁻⁷ g/ml) and such responses were not influenced by BTM-1042 (up to 10⁻⁴ g/ml) but were abolished by tetrodotoxin (3 × 10⁻⁷ g/ml) (Fig. 6). The twitch response of vas deferens to electrical stimulation at 0.1 Hz, which was abolished by tetrodotoxin (3 × 10⁻⁷ g/ml) was inhibited by BTM-1042 in high concentrations (Fig. 7). The twitch response of the phrenic nerve-diaphragm preparation to electrical stimulation, which was abolished by d-tubocurarine (3 × 10⁻⁶ g/ml),

**FIG. 4.** Effects of naloxone on inhibitory actions of BTM-1042 and morphine on the twitch response of guinea pig ileum to electrical stimulation. Similar results were obtained in 6 experiments.

**FIG. 5.** Effects of BTM-1042 and guanethidine on the inhibitory responses of guinea pig taenia caecum to perivascular nerve stimulation (P) and transmural stimulation (T). Similar results were obtained in 8 experiments.
was inhibited by BTM-1042 in high concentrations (Fig. 7). BTM-1042 was more active than procaine in inhibition of responses of the rat vas deferens to electrical stimulation and less active than procaine in inhibition of responses of the rat diaphragm to phrenic nerve stimulation.

Spontaneous movement of the stomach of unanaesthetized rabbits was inhibited by BTM-1042 (0.04 and 0.2 mg/kg i.v.), as shown in Fig. 8. A four-point assay was employed for the spasmyolytic potency ratio, using atropine as the reference drug. The potency ratio of BTM-1042 relative to atropine was 7.4 (95% fiducial limits = 7.9 to 5.1).

DISCUSSION

BTM-1042 had little influence on the responses of the various muscle tissues mediated through the drug-receptors, except for the muscarinic receptor of acetylcholine.
BTM-1042 was less potent than atropine in blocking action of the muscarinic receptor in the ileum from guinea pig but was equipotent with atropine in inhibiting the twitch response of the ileum to electrical stimulation. The responses of guinea pig ileum to the equipotent doses of nicotine and 5-hydroxytryptamine were inhibited by BTM-1042, to the same extent. It has been demonstrated by Nagasawa et al. (7) that, since spontaneous movement of the rabbit stomach in in situ experiments is inhibited by atropine and hexamethonium, the vagus nerve mainly controls movement of the rabbit stomach, in situ. Movement of the stomach of unanaesthetized rabbits was inhibited by BTM-1042 and this drug was about 7 times as potent as atropine, in the in situ experiments. These results suggest that the inhibitory action of BTM-1042 on the twitch response to electrical stimulation is due to inhibition of the release of acetylcholine from the vagus nerve and also to a block of muscarinic receptors. Our results also suggest that inhibition of the acetylcholine-release from the vagus nerve was not due to the narcotic analgesic action of BTM-1042 nor to the stimulation of $\alpha$-adrenoceptors by BTM-1042.

The inability of high concentrations of atropine to inhibit the contractile response of the urinary bladder to parasympathetic nerve stimulation is considered to be evidence in favour of a noncholinergic innervation to this organ (9–11) and it has been suggested that this innervation is also purinergic (11). Since BTM-1042 (up to $10^{-4}$ g/ml) did not influence the contractile response of the urinary bladder to electrical stimulation in the presence of atropine ($3 \times 10^{-7}$ g/ml), this compound probably had no influence on the release of non-cholinergic transmitters in the urinary bladder.

Effective concentrations of BTM-1042 on the contractile responses of the rat vas deferens and rat phrenic nerve-diaphragm to electrical stimulation were $10^{-4}$ to $10^{-3}$ g/ml. When $3 \times 10^{-5}$ g/ml of BTM-1042 was applied to the isolated taenia caecum, 5 out of 8 preparations relaxed with 30–60% of the maximal response. Therefore, we used $10^{-5}$ g/ml of BTM-1042
in these experiments. Since the dose required to block the twitch response of the isolated ileum to electrical stimulation at 0.1 Hz was about $10^{-7}$ g/ml (Fig. 2), it would appear that BTM-1042 had little influence on the inhibitory responses to perivascular nerve stimulation and nonadrenergic inhibitory nerve stimulation, in concentrations required to inhibit the twitch response of the ileum to electrical stimulation. The inhibitory action of BTM-1042 on the release of transmitters from the nerve, such as the motor, sympathetic, and nonadrenergic and noncholinergic nerves seems to be much less than the action on the release of acetylcholine from the vagus nerve in gastrointestinal organs. It can be concluded from the present results that BTM-1042 specifically inhibits the acetylcholine-release from the vagus nerve. It is of interest that the potency of BTM-1042 relative to atropine in the in situ experiments was greater than that seen in the experiments with isolated ileum, and such may indicate that BTM-1042 is a potent antispasmodic for clinical use.

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