SITE OF THE GANGLION BLOCKING ACTION OF GARDNERAMINE AND HIRSUTINE IN THE DOG URINARY BLADDER IN SITU PREPARATION

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Abstract—The ganglion blocking site of gardneramine (GA) and hirsutine (HS) was studied in the dog urinary bladder in an in situ preparation. GA and HS selectively inhibited the DMPP-induced contraction without having an antagonistic effect on the McN-A-343-induced and acetylcholine-induced contraction. In addition, since GA and HS showed a local anesthetic action weaker than that of procaine, the effect of procaine was studied on the same preparation. Procaine inhibited the McN-A-343-induced contraction, and it slightly inhibited the DMPP-induced and acetylcholine-induced contraction. From these findings, it is concluded that GA and HS inhibited the ganglionic transmission of the dog urinary bladder and that the blockade of the nicotinic receptor played a main role.

We studied previously the effect of indole alkaloids contained in the Gardneria genus and the Uncaria genus on the superior cervical ganglionic transmission in cats, rabbits, and rats and on the pelvic ganglionic transmission in guinea pigs and found that gardneramine (GA) and hirsutine (HS) inhibited ganglionic transmission through a mechanism in which blockade of the nicotinic receptor was thought to be most reasonable (1-4). It is generally accepted that in the dog urinary bladder in situ preparation, the parasympathetic ganglia have an excitatory muscarinic receptor that distinct from the nicotinic receptor (5-9). In the present study, the effect of GA and HS on the urinary bladder in situ preparation was examined in order to determine the site of the ganglion blocking action of the alkaloids and also from the viewpoint of species difference. In addition, the effect of procaine was also studied on the same preparation because GA and HS showed a local anesthetic action in an isolated frog sciatic nerve preparation, though the activity of these alkaloids was weaker than that of procaine (4).

Materials and Methods

Female mongrel dogs weighing 6-10 kg were anesthetized with 35 mg/kg of sodium pentobarbital intraperitoneally, and tracheal intubation was performed. A polyethylene tube was inserted in the femoral vein through which an additional anesthetic solution or an appropriate amount of 5% glucose solution was supplied, if necessary. A mid-line laparotomy was performed to expose the urinary bladder. The ureters on both sides were tied and cut, and urine was led outside through a polyethylene tube. The left external iliac artery was freed from the adjacent tissues, and the polyethylene tube was proximally inserted in this artery up to the aorta for drug administration. Both uterine arteries running along the uterus were tied together with the uterus at its lower part.
The abdominal aorta just below the inferior mesenteric artery and the right external iliac artery were freed from the adjacent tissues, and threads were placed around these arteries to be temporarily occluded during the period of drug injection. This procedure permitted the administration of drugs selectively to the bladder. A rubber tube of 6–7 mm diameter was inserted in the urinary bladder through the urethral orifice, and the urinary bladder was filled with saline through the tube after removal of urine. The tube was connected with a strain-gauge low pressure probe (LPU-0.1–350, San-Ei), and the intravesicular pressure was recorded on a pen-writing recorder (056, Hitachi). The hypogastric nerves on both sides were cut peripherally to the inferior mesenteric ganglia. Finally, a saline pool of 37°C was made to protect the operated area from becoming dry. The contraction of the urinary bladder was induced by dimethyiphenylpiperazinium (DMPP), chlorophenylcarbamoyloxybutynyltrimethylammonium (McN-A-343), and acetylcholine (Ach). The doses of DMPP, McN-A-343, and Ach were 10 µg, 50 µg, and 30 µg per animal, respectively. These drugs were applied 20 min before and 3, 30 and 60 min after injection of the test solution. Each of these solutions (0.1–0.2 ml) and a flushing saline containing heparin (150 units/ml) were administered via the intraarterial (i.a.) cannula with a total volume of 0.6 ml and for 20 sec.

Materials: The alkaloids were gardneramine (from Gardneria nutans Sieb. et Zucc.) and hirsutine (from Uncaria rhynchophylla Miq.). The alkaloids were dissolved in 0.1 N phosphoric acid solution, and their pH value was set at 6.2 using 0.2 N sodium hydroxide solution to keep them in a soluble state. Doses of these alkaloids are expressed as the free base per animal. The drugs used were 1,1-dimethyl-4-phenylpiperazinium iodide (Aldrich), 4-(m-chlorophenylcarbamoyloxy)iminostilbene (McNeil Labs), acetylcholine chloride (Daiichi Seiyaku), procaine hydrochloride (Iwaki Seiyaku), hexamethonium bromide (Yamanouchi Seiyaku), and atropine sulfate (Wako). These drugs were dissolved in saline, and their doses refer to those of their salts per animal.

Results

1. Effect of test compounds on urinary bladder contractions induced by McN-A-343 and DMPP: Typical recordings of the effect of the test compounds are presented in Figs. 1–5, and the summarized results are shown in Tables 1 and 2. The phosphoric acid solution of pH 6.2 which served as the control produced a slight inhibitory effect on the McN-A-343-induced contraction in 3 min, while it produced no inhibitory effect on the DMPP-induced contraction (Fig. 1, Tables 1 and 2). GA, 1 and 3 mg/animal, only slightly depressed the McN-A-343-induced contraction, and the same doses of GA depressed the DMPP-induced contraction by 33.9 and 68.8% in 3 min, respectively; and the depressed contractions recovered to the original height in 30–60 min. The potency of the inhibitory effect induced by GA approximately corresponded to that of hexamethonium (Fig. 1, Tables 1 and 2). HS, 1 and 3 mg/animal, augmented the McN-A-343-induced contraction, and the same doses of GA depressed the DMPP-induced contraction by 33.9 and 68.8% in 3 min, respectively; and the depressed contractions recovered to the original height in 30–60 min. The potency of the inhibitory effect induced by GA approximately corresponded to that of hexamethonium (Fig. 1, Tables 1 and 2). HS, 1 and 3 mg/animal, augmented the McN-A-343-induced contraction, while HS, 1 mg/animal, depressed the DMPP-induced contraction by 33.3% in 3 min; and the depressed contraction recovered to the original height in 30 min. On the other hand, HS, 3 mg/animal, produced no inhibitory effect on the DMPP-induced contraction (Fig. 2, Tables 1 and 2). Hexamethonium, 1 and 3 mg/animal, produced a slight inhibitory effect on the McN-A-343-induced contraction in 30 min, with no inhibitory effect in 3 min: and the same doses of hexamethonium depressed the DMPP-induced contraction by 48.8 and
Fig. 1. Effects of gardneramine on the urinary bladder contractions induced by McN-A-343 (upper part) and DMPP (lower part) in dogs. The urinary bladder contractions were induced by intra-arterially applied McN-A-343 (50 μg/animal) and DMPP (10 μg/animal). Control solution (pH 6.2) and gardneramine (1 and 3 mg/animal) were given intra-arterially (i.a.).

Fig. 2. Effects of hirsutine on the urinary bladder contractions induced by McN-A-343 (upper part) and DMPP (lower part) in dogs. The urinary bladder contractions were induced by intra-arterially applied McN-A-343 (50 μg/animal) and DMPP (10 μg/animal). Hirsutine (1 and 3 mg/animal) was given intra-arterially (i.a.).
53.4% in 3 min, respectively, and the depressed contractions recovered to 80% of the original height in 30 min (Fig. 3, Tables 1 and 2). Atropine, 10 and 30 μg/animal, depressed the McN-A-343-induced contraction by 41.0 and 66.2% in 3 min, respectively, and the inhibitory effects of atropine were long lasting and no recovery to 80% of the original height was observed (Fig. 3, Tables 1 and 2).

![Image of Fig. 3](image_url)

**Fig. 3.** Effects of hexamethonium on the urinary bladder contractions induced by McN-A-343 (upper part) and DMPP (lower part) in dogs. The urinary bladder contractions were induced by intra-arterially applied McN-A-343 (50 μg/animal) and DMPP (10 μg/animal). Hexamethonium (1 and 3 mg/animal) was given intra-arterially (i.a.).

**Table 1.** Effects of test compounds on the urinary bladder contraction induced by McN-A-343 (i.a.) in dogs

| Compound     | Dose (mg) | 3 (%) | 30 (%) | 60 (min) | No. of animals |
|--------------|-----------|-------|--------|----------|----------------|
| Control pH 6.2| 1         | 88.4±2.6 | 101.3±9.2 | 94.9±4.4 | 4               |
| Garderamine  | 1         | 88.3±2.1 | 91.6±7.5 | 101.0±3.0 | 5               |
|              | 3         | 81.6±7.8 | 93.5±7.6 | 101.0±3.0 | 5               |
| Hirsutino    | 1         | 117.0±10.5 | 108.2±3.4 | 94.9±4.4 | 4               |
|              | 3         | 136.0±26.8 | 115.7±9.0 | 117.5±14.3 | 4               |
| Hexamethonium| 1         | 95.1±8.4 | 85.7±7.0 | 106.8±8.2 | 4               |
|              | 3         | 98.9±13.2 | 84.8±12.7 | 84.5±14.5 | 4               |
| Atropine     | 0.01      | 59.0±15.1 | 71.4±12.9 | 76.5±9.6 | 5               |
|              | 0.03      | 33.8±2.6 | 88.2±13.3 | 75.4±12.1 | 4               |
| Procaïno     | 1         | 73.8±4.9 | 92.2±4.9 | 93.6±5.8 | 3               |
|              | 3         | 38.6±5.5 | 93.6±5.8 | 93.6±5.8 | 4               |

The urinary bladder contraction was induced by intra-arterially applied McN-A-343 (50 μg/animal). All test compounds were given intra-arterially.
height were observed for 60 min. On the other hand, atropine, 30 μg/animal, produced only a slight inhibitory effect on the DMPP-induced contraction, while a smaller dose of this drug, 10 μg/animal, produced no inhibitory effect (Fig. 4, Tables 1 and 2). Procaine, 1 and 3 mg/animal, depressed the McN-A-343-induced contraction by 26.2

Fig. 4. Effects of atropine on the urinary bladder contractions induced by McN-A-343 (upper part) and DMPP (lower part) in dogs. The urinary bladder contractions were induced by intra-arterially applied McN-A-343 (50 μg/animal) and DMPP (10 μg/animal). Atropine (0.01 and 0.03 mg/animal) was given intra-arterially (i.a.).

Table 2. Effects of test compounds on the urinary bladder contraction induced by DMPP (i.a.) in dogs

| Compound         | Dose (mg) |                |                |                |            |            |            |            |            |
|------------------|-----------|----------------|----------------|----------------|------------|------------|------------|------------|------------|
|                  |           | 3              | 30             | 60 (min)       | No. of animals |
| Control pH 6.2   |           | 98.1±13.6      | 93.5±10.9      | 99.1±2.0       | 4          |
| Gardneramine     | 1         | 66.1±13.1      | 75.8±18.0      | 92.5±11.9      | 4          |
|                  | 3         | 31.2±3.7       | 98.5±12.6      | 96.1±7.4       | 7          |
| Hirsutine        | 1         | 66.7±8.7       | 99.5±17.7      |                | 5          |
|                  | 3         | 90.2±16.4      | 93.9±10.6      | 96.1±7.4       | 7          |
| Hexamethonium    | 1         | 51.2±5.4       | 96.2±13.7      |                | 5          |
|                  | 3         | 46.6±5.7       | 83.1±7.6       | 96.3±1.7       | 6          |
| Atropine         | 0.01      | 91.7±10.8      | 83.4±13.9      | 96.1±7.8       | 4          |
|                  | 0.03      | 72.0±4.6       | 80.4±4.8       | 94.2±6.7       | 4          |
| Procaine         | 1         | 105.7±15.7     | 96.6±15.3      | 93.7±7.8       | 4          |
|                  | 3         | 80.8±19.6      |                |                | 5          |

The urinary bladder contraction was induced by intra-arterially applied DMPP (10 μg/animal). All test compounds were given intra-arterially.
and 61.4% in 3 min, respectively, and the depressed contractions recovered to the original height in about 30 min; but the same doses of procaine produced only a slight inhibitory effect on the DMPP-induced contraction (Fig. 5, Tables 1 and 2).

2. Effect of the test compounds on urinary bladder contraction induced by Ach: The summarized results are shown in Table 3. The control pH 6.2 solution produced no inhibitory effect on the Ach-induced contraction. GA, 3 mg/animal, produced a slight inhibitory effect on the contraction. HS, 1 mg/animal, slightly augmented the con-

Fig. 5. Effect of procaine on the urinary bladder contractions induced by McN-A-343 (upper part) and DMPP (lower part) in dogs. The urinary bladder contractions were induced by intra-arterially applied McN-A-343 (50 µg/animal) and DMPP (10 µg/animal). Procaine (1 and 3 mg/animal) was given intra-arterially (i.a.).

Table 3. Effects of test compounds on the urinary bladder contraction induced by Ach (i.a.) in dogs

| Compound            | Dose (mg) | 3            | 30           | 60 (min)    | No. of animals |
|---------------------|-----------|--------------|--------------|-------------|----------------|
| Control pH 6.2      |           | 96.6±11.1    | 83.4±3.5     | 99.6±9.0    | 5              |
| Gadder amino        | 3         | 80.0±5.8     | 99.2±1.2     |             | 4              |
| Hr urine            | 1         | 104.7±12.1   | 93.1±9.9     | 96.4±3.4    | 8              |
| Hr urine            | 3         | 99.1±4.2     | 92.3±4.7     | 55.4±3.5    | 5              |
| Hexamethonium       | 3         | 88.8±14.5    | 106.9±8.4    |             | 4              |
| Atropine            | 0.01      | 82.2±10.2    | 97.0±5.7     |             | 3              |
| Atropine            | 0.03      | 24.2±7.9     | 33.4±11.2    | 45.1±11.8   | 4              |
| Procaine            | 3         | 73.4±7.5     | 85.2±5.9     |             | 4              |

The urinary bladder contraction was induced by intra-arterially applied Ach (30 µg/animal). All test compounds were given intra-arterially.
traction, while a higher dose of this drug, 3 mg/animal, produced no inhibitory effect. Hexamethonium, 3 mg/animal, produced a slight inhibitory effect on the contraction. Atropine, 10 and 30 μg/animal, depressed the contraction by 17.8 and 78.5% in 3 min, respectively. The inhibitory effect induced by atropine, 30 μg/animal, was long lasting, and the depressed contraction recovered to 54.9% of the original height in 60 min. Procaine, 3 mg/animal, depressed the contraction by 26.6% in 3 min, and the depressed contraction recovered to the original height in 30 min.

Discussion

Taira et al. (5-8) and Saxena (9) reported that in the dog urinary bladder in situ preparation, the DMPP (i.a.)-induced contraction was blocked by hexamethonium and tetrodotoxin, but not by atropine; while the McN-A-343 (i.a.)-induced monophasic fast contraction was blocked by atropine and tetrodotoxin, but not by hexamethonium. From these data, they concluded that the DMPP-induced contraction was due to activation of the nicotinic receptor, while the McN-A-343-induced contraction was due to activation of the muscarinic receptor. In addition, Taira et al. (6) reported that in the same preparation, the contraction induced by Ach (i.a.) was blocked by atropine, but not by hexamethonium and tetrodotoxin, and these workers concluded that the contraction was activated through direct excitation of the muscarinic receptor on the smooth muscle of the urinary bladder.

In the present study, the DMPP (i.a.)-induced contraction was blocked by hexamethonium, but not by atropine; while McN-A-343 in a dose of 50 μg/animal (i.a.) always caused a monophasic fast contraction, and the contraction was blocked by atropine, but not by hexamethonium. These results indicate that the DMPP-induced contraction was produced through excitation of the nicotinic receptor, and the McN-A-343-induced contraction was produced through excitation of the muscarinic receptor on the parasympathetic ganglia of the urinary bladder, selectively. Concerning the active site of Ach in the dose used in the present experiment, as the Ach (i.a.)-induced contraction was blocked by atropine, but not by hexamethonium, the contraction was directly induced through excitation of the muscarinic receptor on the smooth muscle of the urinary bladder. GA, 1 and 3 mg/animal, and HS, 1 mg/animal, selectively inhibited the DMPP-induced contraction, without having an antagonistic effect on the McN-A-343-induced and Ach-induced contractions. As GA and HS showed a local anesthetic action weaker than that of procaine (4), the effect of procaine was studied on the same preparation. Procaine inhibited the McN-A-343-induced contraction, and it slightly inhibited the DMPP-induced and Ach-induced contractions. From these findings, it was indicated that in the dog urinary bladder preparation, GA and HS inhibited the parasympathetic ganglionic transmission through blockade of the nicotinic receptor as a main active site. The decrease in the inhibitory action of HS, 3 mg/animal, on the DMPP-induced contraction may be due to augmentation of the synaptic transmission in the postganglionic nerve terminal and/or of the contractile response on the smooth muscle side, because HS at 3 mg/animal slightly augmented the McN-A-343-induced and Ach-induced contractions and exerted a stimulating effect on spontaneous movement of the urinary bladder in the guinea pig (4). Concerning the effect of procaine, it is well known that procaine inhibits the release of Ach from the nerve terminal (10). Feinstein and Paimre (11, 12) reported that in the isolated smooth muscle preparations of the rabbit taenia coli and guinea pig ileum, their contraction induced by carbachol was non-competitively de-
pressed by local anesthetics such as tetracaine. On the other hand, Kurihara (13) and Kurihara and Sakai (14) reported that procaine enhanced the spontaneous contraction of the isolated guinea pig urinary bladder preparation through a depolarization of the membrane. It is reported that procaine (i.v.) inhibited the ganglionic transmission of the sympathetic and parasympathetic ganglia (15). Smith (16, 17) reported that procaine (i.v.) inhibited the pressor response to McN-A-343 (i.v.) in the cat in situ preparation; and the drug (i.a.) also inhibited the McN-A-343 (i.a.)-induced contraction, but not the DMPP (i.a.)-induced contraction in the cat nictitating membrane in situ preparation. Smith concluded that procaine inhibited the sympathetic ganglionic transmission through blockade of a non-nicotinic receptor, including the muscarinic receptor. In the present study, procaine inhibited the McN-A-343-induced contraction and slightly inhibited the DMPP-induced and Ach-induced contractions. Accordingly, it was concluded that procaine, in the doses used here, inhibited the parasympathetic ganglionic transmission of the urinary bladder through a blockade of the muscarinic receptor with little, if any, effect on the nerve terminal and the smooth muscle.

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