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Central effects of (5*RS*, 1′*SR*)-5-benzyl-3-(3′-morpholino-1′-phenylpropyl)-1, 3-oxazolidin-2-one monofumarate on the function of the bladder and periurethral skeletal muscle in anesthetized rats

Fusako Kagitani [a], Yasunori Gotoh [b], Atsuko Kimura [a], Hitomi Nakayama [a], Akio Sato [a,*]

[a] *Department of the Autonomic Nervous System, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakaecho, Itabashiku, Tokyo-173, Japan*
[b] *Research Laboratory of Nippon Chemiphar Co., Ltd. Misato, Saitama-341, Japan*

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Abstract

The effects of a newly developed drug for incontinence, (5*RS*, 1′*SR*)-5-benzyl-3-(3′-morpholino-1′-phenylpropyl)-1, 3-oxazolidin-2-one monofumarate (NC-1800), on bladder and periurethral skeletal muscle functions were tested in urethane-anesthetized rats. When the bladder pressure was low, i.v. administration of NC-1800 at doses of 4 to 16 mg/kg induced dose-dependent increases of vesical pressure associated with increases in pelvic efferent nerve activity. When the bladder was expanded, the same administration of NC-1800 induced dose-dependent inhibitions of both vesical micturition contractions and rhythmic pelvic burst discharges. Hypogastric efferent nerve activity was not affected. The periurethral electromyogram (EMG) activity was excited when the bladder was contracted, and EMG activity was inhibited when the bladder was relaxed by NC-1800. Pelvic ganglionic transmission, neuromuscular transmission of both bladder and urethra, and muscle contractility itself of bladder and urethra were not affected by NC-1800. These results suggest that NC-1800 modulates the functions of the bladder and urethra by influencing pelvic and pudendal nerve activity via the central nervous system. © 1997 Elsevier Science B.V.

Keywords: Bladder; Pelvic nerve; Micturition contraction; Periurethral muscle; Pudendal nerve; (Rat)

1. Introduction

In a previous study, we examined the effect of the centrally acting muscle relaxant, (4*S*,5*R*)-4-(2-methylpropyl)-3-[3-(perhydroazepin-1-yl) propyl]-5-phenyl-1,3-oxazolidin-2-one (NC-1200), on the micturition contractions of the urinary bladder in anesthetized rats (Kimura et al., 1988). We showed that NC-1200 inhibited the micturition contractions by inhibiting pelvic efferent nerve activity and that this inhibitory effect originated mainly in the central nervous system. Systemic administration of NC-1200 simultaneously induced a dose-dependent decrease in blood pressure. The depressor effect of NC-1200 limits its clinical application for treatment of disorders of micturition. Recently, a new drug, (5*RS*,1′*SR*)-5-benzyl-3-(3′-morpholino-1′-phenylpropyl)-1, 3-oxazolidin-2-one monofumarate (NC-1800) (Fig. 1), which is an analogue of NC-1200 and has no depressor effect, was developed by Nippon Chemiphar. NC-1800 was shown to inhibit the micturition contractions of the bladder in anesthetized animals (Masaki et al., 1995). It was suggested that NC-1800 could be used for the treatment of urinary disorders. However, the neural mechanism of the effect of NC-1800 on the micturition contractions of the bladder has not been resolved. The present study aimed to examine the effects of NC-1800 on the activity of the bladder and periurethral sphincter muscles and to clarify its neural mechanism using anesthetized rats.

For this purpose, at first the effect of NC-1800 on the bladder and periurethral sphincter muscles was examined both when the bladder volume was small, and also when the bladder was expanded to produce rhythmic micturition contractions. Secondarily, we studied the effects of NC-

* Corresponding author. Tel.: (81-3) 3964-3241, ext. 3087; Fax: (81-3) 3964-1415; e-mail: satoakio@center.tmig.or.jp

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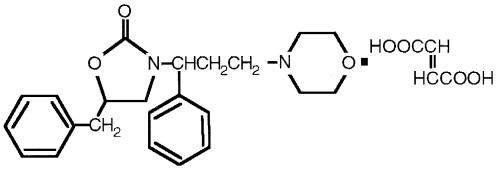

Fig. 1. Chemical structure of NC-1800.

1800 on parasympathetic pelvic and sympathetic hypogastric nerve activity to the bladder and on nerve-stimulation-induced responses of bladder and urethra.

2. Materials and methods

Experiments were performed on 22 adult Wistar rats (190–430 g) anesthetized with urethane (1.1 g/kg s.c.). Respiration was maintained through a tracheal cannula connected to an artificial respirator (SN-480-7, Shinano, Japan). The end-tidal CO_2 concentration, monitored with a gas analyzer (1H26, NEC San-ei, Japan), was kept at about 3.0%. Arterial blood pressure was continuously recorded from a catheter kept in a common carotid artery. During the experiment, the depth of anesthesia was routinely judged by observing the animal's motion, respiration, blood pressure and heart rate. Whenever these conditions were unstable, additional doses of urethane (0.05–0.1 g/kg) were given intravenously through a catheter kept in an external jugular vein. Rectal temperature, monitored by a thermistor, was maintained between 37.0 and 38.0°C with a temperature-control-device including a thermostatically-regulated infrared lamp and direct current heat pad (ATB-1100, Nihon Kohden, Japan).

We used the vesical balloon method (Kimura et al., 1988) or urethral catheter method (Sato et al., 1992) for vesical pressure recording. The abdomen was opened about 6 cm just above the pubic bone by midline section. A small balloon made of condom rubber connected to a polyethylene tube was inserted into the urinary bladder from a small incision made at the vesical apex, or a polyethylene catheter was inserted into the urinary bladder about 2.5 cm from the urethral opening. The balloon and/or the bladder was filled with warm saline so that the basic vesical pressure could be kept within the desired levels. Intravesical pressure was measured by a transducer (TP-200T, Nihon Kohden) through a vesical balloon or a urethral catheter and recorded on a polygraph (RM-6000, Nihon Kohden). In all experiments the ureters were kept intact. The rats were kept in the supine position during the experiment. The pelvic cavity was kept open and covered with paraffin oil to prevent evaporation of fluid and to maintain the vesical temperature at body temperature with the help of an infrared lamp.

The muscles overlying the pubic symphysis were separated and the cartilage of the pubic symphysis was divided to expose the prostate and urethra. Distension of the bladder to a pressure of 200 mmH_2O or above caused twitching of some skeletal muscle in the proximal urethra. Fine bipolar needle wire (diameter 120 μm) electrodes were inserted into this muscle so that electromyographic (EMG) activity of the periurethral sphincter muscles could be recorded. EMG activity was recorded using a preamplifier using a time constant of 0.01 s (S-0476, Nihon Kohden). Spikes of EMG activity were counted every 1–2 s with a spike counter (ATAC-3700, Nihon Kohden), and recorded on a polygraph (RM-6000, Nihon Kohden).

Parasympathetic postganglionic vesical nerve branches coming from the pelvic ganglion located on the surface of the prostate, and sympathetic postganglionic vesical nerve branches of the hypogastric nerves were dissected under a binocular microscope for the recording of their activities. Mass discharges of their efferent nerve branches were recorded from the central cut segment of the nerve branches with bipolar platinum–iridium wire electrodes. Nerve activity was recorded inside a warm liquid paraffin pool, and was amplified by a preamplifier (S-0476, Nihon Kohden) using a 0.01 s time constant. The nerve activity was led to a window discriminator which passed only discharges greater than background noise levels. The number of nerve discharge spikes was counted every 2 s by a spike counter (ATAC 3700, Nihon Kohden), and recorded on a polygraph (RM-6000, Nihon Kohden).

The pelvic preganglionic nerve was dissected to about 2 cm in length and cut centrally. Single shock (0.5 ms pulse width, supramaximal 5–10 V intensity) stimulations were delivered to the peripheral end of the severed pelvic preganglionic nerve by an electrical stimulator (SEN-7103, Nihon Kohden). Evoked potentials were recorded from the pelvic postganglionic nerve through a preamplifier using a time constant of 0.3 s. The evoked potentials were averaged 10 times by the averaging instrument (ATAC-3700, Nihon Kohden).

Electrical stimulation (0.5 ms pulse width, supramaximal 5–10 V intensity, 20 Hz frequency, 5 s stimulus duration) was delivered to the peripheral end of the severed pelvic preganglionic nerve and changes in vesical pressure were recorded to test neuromuscular transmission to the bladder.

The pudendal nerve was dissected and single shock (0.1 ms pulse width, 0.1–1 V intensity) stimulations were delivered to the peripheral end of the pudendal nerve. The evoked EMG was recorded from the urethral muscle (time constant, 0.3 s) and averaged 10 times by the averaging instrument.

NC-1800 (Nippon Chemiphar, Japan) was dissolved in saline at a concentration of 8 mg/ml, and administered i.v. at doses of 0.025–0.2 ml/100 g body weight (injection time 30–60 s). Four different doses (2, 4, 8 and 16 mg/kg) were injected, from the smaller dose to the larger dose. We usually waited about 10–30 min between each trial, or waited until all effects of the drug on the bladder disappeared. A ganglionic blocker (hexamethonium bro-

mide: C6, Yamanouchi, Japan) and a skeletal muscle relaxant (gallamine triethiodide, Sigma) were dissolved in saline at a concentration of 40 and 20 mg/ml, respectively, and were administered i.v. at doses of 20 mg/kg.

All data were expressed as mean ± S.E.M. The data were statistically analyzed by repeated measures of analysis of variance (ANOVA) followed by Dunnett's least-significant-difference test for correction of multiple comparisons or Student's paired *t*-test.

3. Results

3.1. Effects of NC-1800 on bladder pressure and periurethral sphincter muscle activity when the bladder volume was small

The intravesical balloon was filled with a small volume of warm saline (0.1–0.2 ml), so that intravesical pressure reached 30–100 mmH_2O and caused small, irregularly fluctuating contractions of the bladder. These small fluctuating contractions of the bladder have been shown to be of myogenic origin, independent of extrinsic vesical autonomic nerve activity (Sato et al., 1975). This state of the bladder with small fluctuations at a small volume was referred to as the quiescent bladder (Sato et al., 1975). Fig. 2A shows sample records (in 1 rat) and Fig. 2B shows summarized data (in 5 rats) of the effects of an i.v. bolus administration of 8 mg/kg NC-1800 on the quiescent bladder and on the periurethral EMG activity. The intravesical pressure and the periurethral EMG activity both

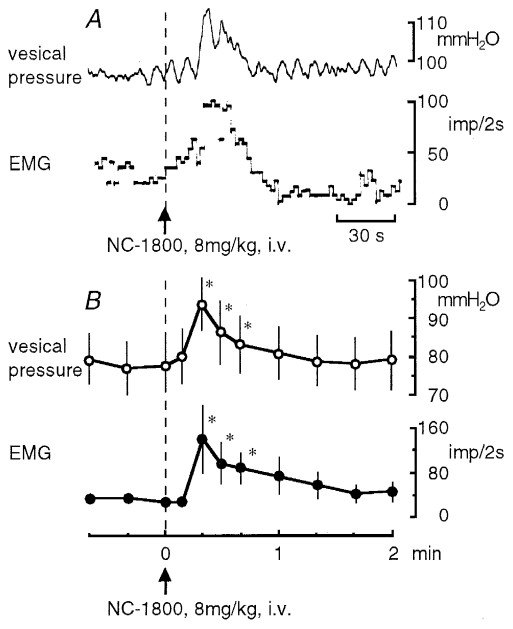

Fig. 2. Effects of NC-1800 administration (8 mg/kg i.v.) on vesical pressure and periurethral EMG activity. (A) Specimen records. (B) Averaged responses in 5 rats. * $P < 0.05$; significantly different from the values without the drug.

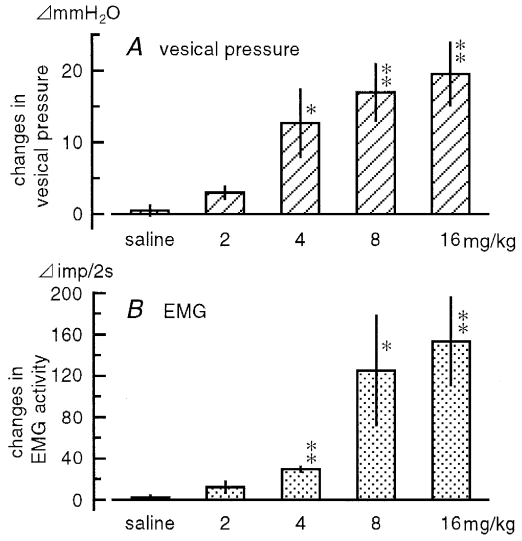

Fig. 3. Effects of various doses of NC-1800 on vesical pressure (A) and periurethral EMG activity (B). Changes in vesical pressure and EMG activity induced by NC-1800, compiled from 5 animals tested. * $P < 0.05$, ** $P < 0.01$; significantly different from the values after saline injection.

increased significantly for about 30 s starting 20 s after i.v. injection of NC-1800. The maximum increase of intravesical pressure from the basal pressure was about 17 ± 4 mmH_2O.

Fig. 3 summarizes the effects of various doses of NC-1800 (from 2 to 16 mg/kg) on bladder pressure and periurethral EMG activity in the quiescent bladder in 5 rats. A dose of 2 mg/kg was not effective, but 4–16 mg/kg increased both the intravesical pressure and periurethral EMG activity in a dose-dependent manner. The threshold dose for this excitation was 4 mg/kg.

We examined whether NC-1800 influenced not only the periurethral muscles, but other skeletal muscles, e.g., hindlimb muscles, by monitoring hindlimb EMG activity in 3 rats. When NC-1800 (8 mg/kg, i.v.) increased both vesical pressure and the periurethral EMG activity, hindlimb EMG activity was not influenced at all.

The effects of the skeletal muscle relaxant (gallamine triethiodide, 20 mg/kg i.v.) on NC-1800-induced responses of vesical pressure and periurethral EMG activity were examined. Intravenous administration of the neuromuscular blocker did not affect the NC-1800-induced vesical contraction, but abolished the NC-1800-induced periurethral EMG activity.

A typical example of the effect of NC-1800 on both pelvic and hypogastric efferent nerve activities, recorded simultaneously with the intravesical pressure in the same animal, is shown in Fig. 4. This recording shows that i.v. administration of NC-1800 (16 mg/kg) produces increases in both pelvic efferent nerve discharges and vesical pressure (Fig. 4A), but does not affect hypogastric nerve activity (Fig. 4B). Results similar to this example were obtained in 3 other rats.

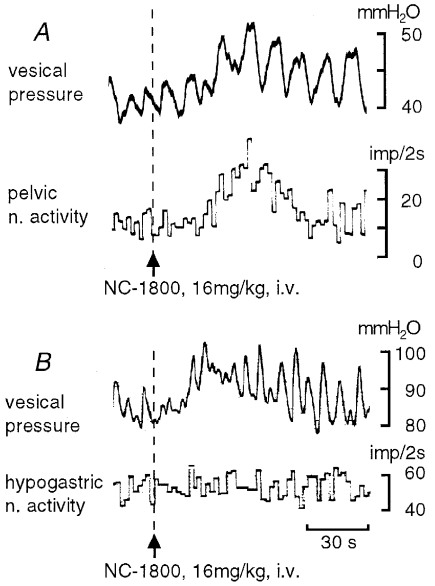

Fig. 4. Effects of NC-1800 administration (16 mg/kg i.v.) on the intravesical pressure with simultaneous recording of efferent nerve discharge of the pelvic and hypogastric nerve branches. Injection of NC-1800 is indicated by the arrow. Efferent nerve discharges: counts per 2 s.

3.2. Effects of NC-1800 on the bladder pressure and periurethral sphincter muscle activity when the bladder was expanded (causing rhythmic micturition contractions)

When the bladder was inflated with an appropriate amount of saline, and the intravesical pressure was set between 180–340 mmH_2O, the bladder showed large rhythmical micturition contractions with an amplitude of 150–600 mmH_2O and a frequency of 1–2/min (Fig. 5A and 6 before drug injection). Each micturition contraction was produced by a corresponding burst of discharge of the pelvic efferent nerve (De Groat and Ryall, 1969; Sato et al., 1975, 1977, 1980). During rhythmic micturition contractions, the periurethral sphincter muscles changed their EMG activity. When the bladder was relaxed, their EMG activity was continuous (Fig. 5B). But when the bladder produced micturition contractions, the EMG activity showed an oscillatory rhythm, particularly during the rising phase of the micturition contraction (Fig. 5C). The oscillatory rhythmic activity of the EMG was composed of alternatively active and silent phases, each of which lasted for 80–270 ms (Fig. 5C) as described by Morrison et al. (1995). The rhythmic activity of the EMG during a micturition contraction showed an increased pattern of activity when counted by the spike counter every 2 s as shown in Fig. 6.

Representative simultaneous recordings of the rhythmic micturition contractions of the bladder and oscillatory rhythmic firing in the periurethral EMG following i.v. injection of NC-1800 are shown in Fig. 5. The rhythmic micturition contractions of the bladder and oscillatory rhythmic firings in the periurethral EMG were inhibited for about 10 min following i.v. administration of NC-1800 (8 mg/kg). However, tonic discharges of the periurethral EMG were maintained during the NC-1800-induced inhibition of bladder contractions. Fig. 6 shows some examples of the effects of various intravenous doses (2–16 mg/kg) of NC-1800 on vesical micturition contractions and peri-

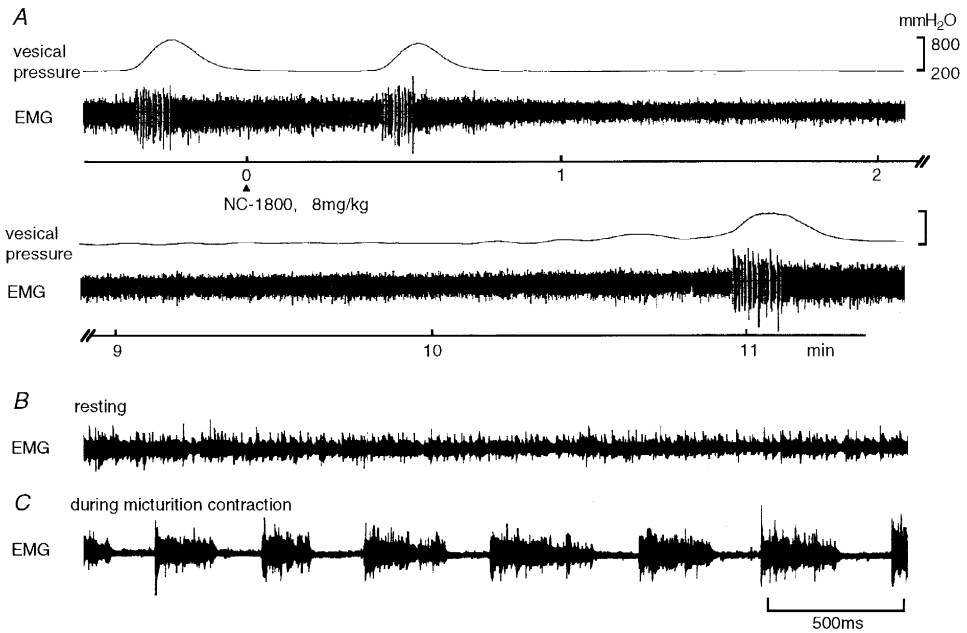

Fig. 5. (A) Effects of NC-1800 administration (8 mg/kg i.v.) on intravesical pressure and periurethral EMG activity during rhythmic micturition contractions. Intravesical pressure and periurethral EMG activity are recorded on visicorder in one rat. Injection of NC-1800 is indicated by the triangle. (B and C) Traces of EMG activity from periurethral striated muscle during resting (B) and during the rising phase of the micturition contraction (C) before drug injection recorded in another rat. The time scale is expanded so as to show the detail.

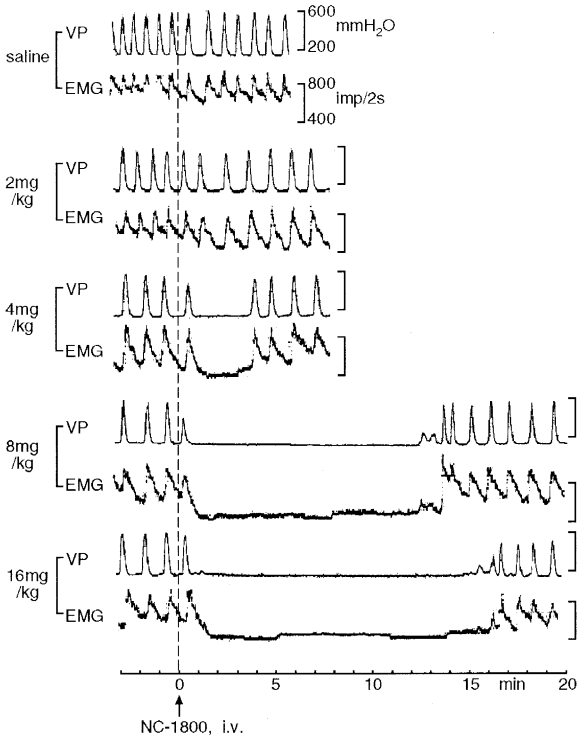

Fig. 6. Sample recordings of the large rhythmic micturition contractions of the bladder (VP) and periurethral EMG activity, and the effects of various doses of i.v. administered NC-1800 on these contractions and EMG activity in one rat. EMG activity: counts per 2 s.

urethral EMG activity in one rat. A dose of 2 mg/kg of NC-1800 was ineffective, but 4–16 mg/kg inhibited both the vesical micturition contractions and oscillatory EMG activity in a dose-dependent manner. The period of these inhibitions was about 2 min with 4 mg/kg of NC-1800, but was prolonged to about 12 min with 8 mg/kg of NC-1800, and reached about 14 min with 16 mg/kg of NC-1800. Results similar to those shown in Fig. 6 were obtained in 4 other rats.

The effect of NC-1800 (8 mg/kg i.v.) on pelvic and hypogastric efferent nerve activity, recorded simultaneously with the vesical micturition contractions in the same animal, are demonstrated in Fig. 7. As shown in this figure, the rhythmic vesical micturition contractions were

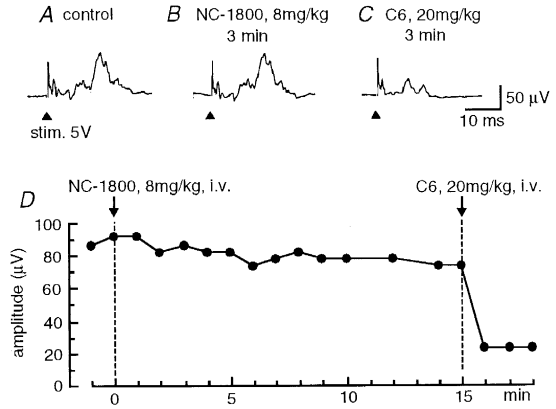

Fig. 8. Effects of i.v. administration of NC-1800 and hexamethonium bromide (C6) on pelvic ganglionic transmission. (A–C) Sample recordings of evoked potentials in the pelvic postganglionic nerve to stimulation of the pelvic preganglionic nerve. (D) Time-course of amplitude of late evoked potential before and after NC-1800 and C6 administration. A single shock stimulation of 5 V intensity delivered every 3 s at the arrow, responses averaged 10 times.

accompanied by the rhythmic burst discharges of the postganglionic vesical branches of the pelvic nerve, but postganglionic vesical branches of the hypogastric nerve usually did not show any rhythmic burst discharges corresponding to the vesical contractions. Intravenous administration of NC-1800 (8 mg/kg) inhibited both the rhythmic burst discharges in the pelvic nerves and vesical contractions for about 12 min without any significant effect on hypogastric efferent nerve activity. Similar results were obtained in 3 other rats.

3.3. Effects of NC-1800 on synaptic transmission at the pelvic ganglion and neuromuscular junction

The effect of i.v. administration of NC-1800 on synaptic transmission at the pelvic ganglion from preganglionic to postganglionic neurons innervating the bladder was tested in 4 rats (Fig. 8).

A single electrical stimulus to a pelvic preganglionic nerve evoked two kinds of potentials in pelvic postganglionic nerves; one with a short latency and the other with a long latency. A typical example is shown in Fig. 8A, in

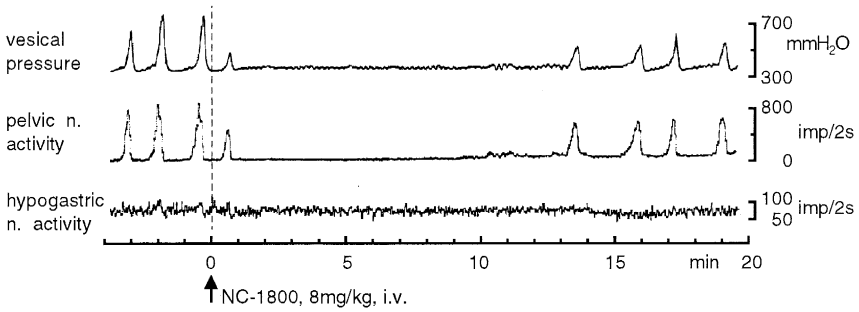

Fig. 7. Effects of NC-1800 administration (8 mg/kg i.v.) on rhythmic micturition contractions with simultaneous recording of efferent nerve discharges of the pelvic and hypogastric nerve branches. Injection of NC-1800 is indicated by the arrow. Efferent nerve discharges: counts per 2 s.

which the early response had a latency of 1 ms, and the late one had a latency of 8 ms. Intravenous administration of NC-1800 (8–16 mg/kg) did not have a significant effect on these two potentials (Fig. 8B). The i.v. administration of 20 mg/kg C6, known to block synaptic transmission at the ganglion at this dose (Borchard et al., 1990), markedly depressed the late potential (Fig. 8C) whereas the early one was not affected. The potentials that did not disappear even after C6 administration (early potential and marginal late potential) occurred when the stimulating and recording electrodes were reversed. The remained responses are thought to be direct action potentials of afferent fibers passing through the ganglion, and most of the late one is thought to be synaptically transmitted at the ganglion (Kimura et al., 1988).

Electrical stimulation at supramaximal intensity (10 V, 20 Hz, 5 s) of efferent fibers of a pelvic nerve proximal to the pelvic ganglion elicited bladder contractions. The amplitude of bladder contractions elicited by pelvic efferent nerve stimulation was not affected by NC-1800 (8 mg/kg, i.v.) when tested in 4 rats.

Single electrical stimulation of efferent fibers of a pudendal nerve elicited a periurethral sphincter EMG response. When the stimulus strength was gradually increased, the EMG response first started to appear at 0.2 V and increased intensity-dependently and reached to the maximum at 0.5 V. The EMG response elicited by pudendal nerve stimulation (1 V) was not affected by NC-1800 (8 mg/kg, i.v.), whereas i.v. administration of 20 mg/kg gallamine triethiodide, a skeletal muscle relaxant, completely depressed the pudendal nerve stimulus-induced periurethral sphincter EMG response.

3.4. Direct effects of NC-1800 on the bladder and periurethral sphincter muscles

In 4 rats, the direct effects of NC-1800 on the bladder and periurethral sphincter muscles were examined after bilateral severance of both pelvic and pudendal nerves innervating the bladder and urethral sphincter muscles. There were no effects on vesical pressure and the periurethral EMG activity following i.v. administration of NC-1800 (8 mg/kg) after bilateral severance of pelvic and pudendal nerves.

3.5. Effects of NC-1800 on systemic arterial blood pressure

In all 22 cases examined in the present experiments, i.v. administration of NC-1800 (2–8 mg/kg) had no significant effects on systemic arterial blood pressure. Administration of 16 mg/kg NC-1800 decreased systemic arterial blood pressure maximally by 12 ± 3 mmHg when observed for 30 min after the injection.

4. Discussion

The present experiments demonstrated that in anesthetized rats i.v. administration of NC-1800 had either an excitatory or inhibitory effect on both bladder contractility and urethral sphincter activity depending on the bladder condition, expanded or quiescent. The effect was excitatory if the intravesical pressure was low, and the effect was inhibitory if the intravesical pressure was high.

The excitatory effect of i.v. administration of NC-1800 on vesical pressure and periurethral EMG activity at low intravesical pressure, disappeared after pelvic and pudendal nerve severance. Intravenous administration of NC-1800 increased activity of postganglionic vesical branches of pelvic efferent nerves, but not the activity of hypogastric nerves. Intravenous administration of NC-1800 did not affect contractility of smooth muscles of the bladder, electrical activity of sphincter muscles of the urethra, neuromuscular transmission from the pelvic efferent nerve to the bladder detrusor muscles, neurotransmission from the pudendal efferent nerve to the urethral sphincter muscles, and synaptic transmission at the pelvic ganglion from the pelvic preganglionic neurons to the postganglionic neurons innervating the bladder. Therefore, it was concluded that the excitatory effect of NC-1800 on bladder contractility and periurethral sphincter muscle activity at low vesical pressure was due to increases in pelvic efferent nerve activity and pudendal efferent nerve activity, and it was assumed that the site of this excitatory action of NC-1800 on these efferent nerve fibers was somewhere in the central nervous system.

The inhibitory effect of i.v. administration of NC-1800 on rhythmic vesical micturition contractions and the corresponding rhythmic EMG activity in the periurethral sphincter muscles seen during expansion of the bladder in anesthetized rats was always accompanied by disappearance of rhythmic burst discharges in the postganglionic vesical branches of pelvic efferent nerves, which are known to be responsible for production of the rhythmic micturition contractions (De Groat and Ryall, 1969; Sato et al., 1975, 1977, 1980). As NC-1800 had no effect on the activity of the vesical hypogastric efferent nerve, sympathetic nerves could not be involved in the NC-1800-induced inhibition of micturition contractions. It is suggested that NC-1800 administered intravenously acts on the central nervous system and inhibits the micturition contractions by inhibiting rhythmic activity of pelvic efferent nerves and also inhibits burst discharges of the periurethral sphincter muscles. The inhibition of burst discharges of the periurethral sphincter muscles is due to the inhibition of burst discharges of pudendal efferent nerves, because NC-1800 did not act on synaptic transmission at the periurethral neuromuscular junction. It remains uncertain if NC-1800-induced inhibition of a pudendal efferent nerve was elicited by a direct inhibitory action of NC-1800 on the pudendal neurons or by a secondary action due to the

disappearance of pelvic burst discharges which were initially elicited by NC-1800.

NC-1800 inhibits the rhythmic burst discharges of the periurethral EMG, while it maintains the tonic activity of the EMG. Thus, NC-1800 seems to have an overall inhibitory effect on voiding of urine, because the urethra maintains its muscle tone to a certain degree and the bladder stops contracting with NC-1800 injection.

The cell bodies of the preganglionic neurons of the pelvic nerves exist in the intermediate gray matter of the sacral spinal cord (Nadelhaft et al., 1980), and activity of these neurons is under the control of the supraspinal neural structures. The generation of rhythmic activity of the pelvic efferent nerve producing the rhythmic vesical micturition contractions has been shown to originate in the brainstem in anesthetized animals with an intact central nervous system (Barrington, 1914, 1928; Tang and Ruch, 1956; Ruch, 1960; Kuru, 1965; Edvardsen, 1968; De Groat and Ryall, 1969). In rats, Satoh et al. (1978a,b) have located the detrusor center to the dorsolateral tegmental nucleus. Therefore, it can be suggested that NC-1800 excites or inhibits pelvic efferent nerves in the central nervous system, perhaps the detrusor center in the brainstem or at the sacral spinal cord. The precise level of the central action of NC-1800, for example at the brainstem or at the spinal cord remains uncertain at this moment.

NC-1800 administered intravenously at doses between 2 and 8 mg/kg did not affect systemic blood pressure although NC-1200, an analogue of NC-1800 had a potent hypotensive effect on systemic blood pressure (about 30 mmHg decrease at 10 mg/kg i.v., Kimura et al., 1988). The clinical use of NC-1200 was withheld because of its hypotensive effect. NC-1800 has no obvious hypotensive effects, but has a significant effect on the bladder and periurethral sphincter muscles, either excitatory or inhibitory depending on whether the bladder is expanded or not expanded. Therefore, NC-1800 could be tested for regulation of micturition functions.

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