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# Effects of Verapamil and Sodium Nitroprusside on Acetylcholine-induced Contraction of the Rabbit Detrusor Muscle

M. Gotoh[1], A. Kondo[1], M. Hassouna[2] and M. M. Elhilali[2]

[1] Department of Urology, Nagoya University School of Medicine, Nagoya, Japan
[2] Department of Urology, Royal Victoria Hospital, McGill University, Montreal, Canada

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**Summary.** Effects of extracellular and intracellular $Ca^{2+}$ on acetylcholine-induced contraction of the bladder detrusor muscle were studied in vitro, utilizing two types of $Ca^{2+}$ antagonists of different mechanisms of action; verapamil and sodium nitroprusside (NP). Acetylcholine ($10^{-8}$ to $10^{-2}$ M) caused dose-dependent contractions of the detrusor muscle strips. Pretreatment of the strips with verapamil ($10^{-7}$, $10^{-6}$ M) significantly inhibited the acetylcholine-induced contraction in a dose-dependent manner, whereas NP ($10^{-7}$ to $10^{-5}$ M) failed so suppress the contraction. The contraction of the strips once elicited by acetylcholine ($10^{-6}$ M) could be completely relaxed by verapamil ($10^{-5}$ M) addition, but only incompletely by NP ($10^{-5}$, $10^{-4}$ M). In $Ca^{2+}$-free solution containing 0.01 mM EGTA, replenishment of $Ca^{2+}$ (2.5 mM) to the medium caused contractions of the strips. Addition of acetylcholine ($10^{-6}$ M) to the medium enhanced the $Ca^{2+}$-induced contraction, which was significantly inhibited by pretreatment with verapamil ($10^{-6}$ M), but not affected by NP ($10^{-6}$ M). In $Ca^{2+}$-free medium containing 0.1 mM EGTA, acetylcholine caused a slight degree of tension increase of the strips in a dose-dependent fashion, at higher concentrations exceeding $10^{-6}$ M. These results suggest that the detrusor muscle contraction induced by acetylcholine is mostly dependent of extracellular $Ca^{2+}$ influx both in its initiation and maintenance. It is also supposed, however, that intracellular $Ca^{2+}$ fractions will partly participate in the acetylcholine-induced contraction and possibly in its maintenance.

**Key words:** Bladder detrusor muscle, Acetylcholine, Calcium.

## Introduction

Contraction-relaxation cycles in smooth muscle are dependent on the regulation of free $Ca^{2+}$ in the myoplasm as is the case in skeletal muscle [3]. Excitation-contraction coupling of skeletal muscle practically is insensitive to changes in extracellular $Ca^{2+}$ concentration or transmembrane $Ca^{2+}$ influx, since intracellular stores in the sarcoplasmic reticulum provide sufficient amount of $Ca^{2+}$ to achieve activation of the contractile system. By contrast, the intracellular $Ca^{2+}$ stores of smooth muscle cell are thought to be rather limited [12], and also morphological basis for storage and release of $Ca^{2+}$ in this type of muscle could not be demonstrated as clearly as in the skeletal muscle system. It is generally suspected, that $Ca^{2+}$ has to be rapidly refilled from extracellular sources during mechanical activity of smooth muscle. In some types of smooth muscles, in particular those of blood vessels, the relationship of agonist-induced contraction with $Ca^{2+}$ flux have been recently studied using various kinds of $Ca^{2+}$ antagonists [2, 7]. On bladder detrusor muscle, however, there have been a limited number of reports on the relationship between agonist-induced contraction and $Ca^{2+}$ [1, 4, 8, 11]. In the present study, we investigated the interactions of acetylcholine with extracellular and intracellular $Ca^{2+}$ on rabbit bladder detrusor muscle, comparing the differential effects of two types of $Ca^{2+}$ antagonists of different mechanisms of action (verapamil and sodium nitroprusside) on acetylcholine-induced contraction. Verapamil is one of $Ca^{2+}$ entry blockers capable of selective inhibition of transmembrane $Ca^{2+}$ influx from the extracellular spaces [15], while sodium nitroprusside inhibits the activation of intracellular $Ca^{2+}$ independent of extracellular $Ca^{2+}$ uptake [7, 14].

## Materials and Methods

### *Tissue preparations*

Adult male New Zealand rabbits weighing 2 to 3 kg were sacrificed by a blow to the neck followed by exsanguination. The bladders

*Correspondence and requests for reprints:* Momokazu Gotoh, M.D., Department of Urology, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, 466 Nagoya, Japan

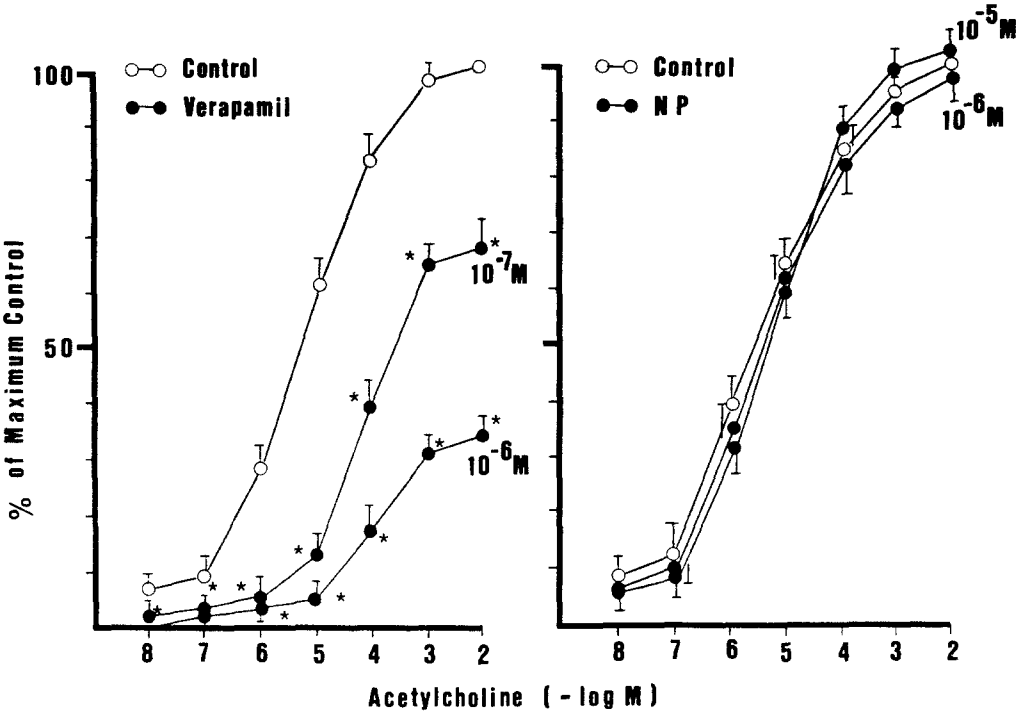


**Fig. 1.** Effects of verapamil and sodium nitroprusside (NP) on acetylcholine-induced contraction of detrusor muscle strips. Curves represent mean ± SE responses from 8 strips in each experiment. 100% = 5.1 ± 0.3 g. (* Significantly different from control at $p < 0.01$)

were immediately dissected and placed in Krebs' solution containing (in mM) NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $KH_2PO_4$ 1.1, $NaHCO_3$ 25 and glucose 11. Smooth muscle strips of equal length (1 cm) were obtained from the bladder body after removal of the mucosa. The detrusor strip was suspended in a bath containing Krebs' solution at 37 °C which was continuously perfused with a gas mixture of 95% oxygen and 5% carbon dioxide. A 30 min accommodation period was allowed for the strips to develop their inherent motility before applying a resting tension of 1 g and further 30 min were allowed for equilibration to the tension. Tension measurements were performed with a Grass FT03 force displacement transducer and recorded on a Grass polygraph. $Ca^{2+}$-free Krebs' solution containing 0.01 or 0.1 mM Ethyleneglycol-bis-N,N'-tetra-acetic acid (EGTA) was used in some experiments.

*Experimental Protocol*

(1) After equilibration, the first group of experiments were performed to study the effects of verapamil or sodium nitroprusside (NP) on the acetylcholine-induced contraction of the detrusor strips. Each strip was first challenged with acetylcholine in a cumulative way ($10^{-8}$ to $10^{-2}$ M), and the tension changes of the strip were observed for 3 min following each addition. After washing the strip and following a 60 min recovery period for reequilibration, either verapamil or NP was added to the bathing medium at the concentration of $10^{-7}$ M and allowed to act for 5 min before cumulative addition of acetylcholine ($10^{-8}$ to $10^{-2}$ M). The same procedure was repeated on higher concentrations of verapamil ($10^{-6}$ M) or NP ($10^{-6}$, $10^{-5}$).

(2) The second group of experiments were performed using $Ca^{2+}$-free Krebs' solution containing 0.01 mM EGTA. After equilibration and establishment of inherent motility of fresh strips in the normal Krebs' solution, the bathing medium was changed to a $Ca^{2+}$-free solution. After incubation of the strips for 30 min in this solution, the medium was replenished with 2.5 mM $Ca^{2+}$ and the tension changes of the strips were observed for 5 min. After washing and 30 min recovery period in the $Ca^{2+}$-free solution, $10^{-6}$ M acetylcholine was added to the bathing medium and allowed to act for 2 min before the replenishment of $Ca^{2+}$ (2.5 mM). After washing and waiting for 30 min in the $Ca^{2+}$-free medium, verapamil or NP ($10^{-6}$ M) was added to the medium. 5 min later, $10^{-6}$ M acetylcholine was also added to the medium and allowed to act for 2 min before the $Ca^{2+}$ (2.5 mM) replenishment.

(3) In the third group of experiments, after equilibration of fresh muscle strips in the normal Krebs' solution, the bathing medium was changed into $Ca^{2+}$-free Krebs' solution containing 0.1 mM EGTA. After incubation of the strips for 30 min in this solution, acetylcholine was added to the medium cumulatively at concentrations of $10^{-8}$ to $10^{-3}$ M. After washing and reequilibration in the $Ca^{2+}$-free medium, verapamil, NP or atropine ($10^{-6}$ M) was added to the medium and allowed to act for 5 min before the cumulative addition of acetylcholine ($10^{-8}$ to $10^{-3}$ M).

(4) The final set of experiments studied the relaxing effects of verapamil and NP on the detrusor muscle strips contracted by acetylcholine. Various concentrations of verapamil, NP or atropine were added to the strips contracted by acetylcholine ($10^{-6}$ M) in the normal Krebs' solution (relaxation study).

The drugs used were acetylcholine (Sigma), atropine (IMS), verapamil hydrochloride (Searle), sodium nitroprusside (Fisher) and EGTA (Fisher). All concentrations given are final concentrations in the bath. The tension changes of the preparations were converted to the percentage of the maximum response and data presented as mean ± SE. Statistical comparisons were made using Student's *t*-test.

## Results

Figure 1 shows the dose-response curves to the cumulative acetylcholine addition in the normal Krebs' solution, under normal condition (control) and in the presence of verapamil ($10^{-7}$, $10^{-6}$ M) or NP ($10^{-6}$, $10^{-5}$ M). In the control, the cumulative addition of acetylcholine ($10^{-8}$ to $10^{-2}$ M)

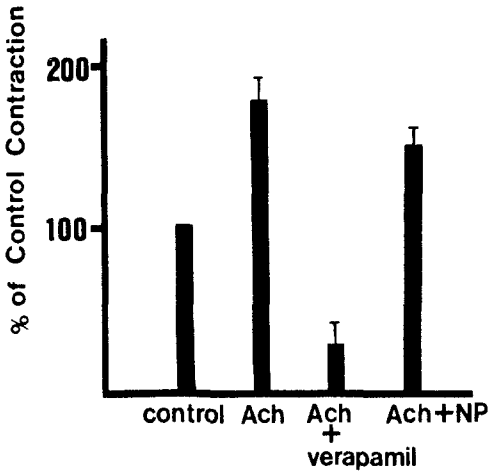


Fig. 2. Effects of acetylcholine, verapamil and sodium nitroprusside (NP) on $Ca^{2+}$ (2.5 mM)-induced contraction of detrusor muscle strips in $Ca^{2+}$-free Krebs' solution containing 0.01 mM EGTA. $Ca^{2+}$-induced contraction without drugs (control), in the presence of acetylcholine ($10^{-6}$ M), acetylcholine ($10^{-6}$ M) plus verapamil ($10^{-6}$ M) and acetylcholine ($10^{-6}$ M) plus NP ($10^{-6}$ M) are compared. 100% = 0.5 ± 0.1 g. ($n$ = 8 in each experiment)

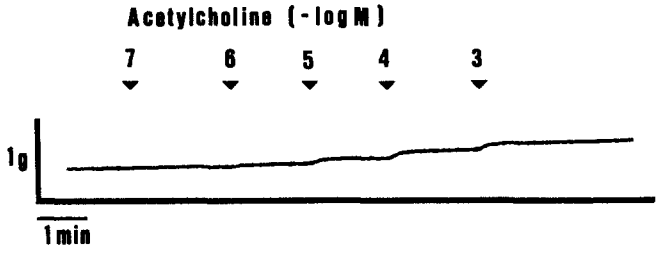


Fig. 3. Responses of detrusor muscle strips to cumulative acetylcholine addition in $Ca^{2+}$-free Krebs' solution containing 0.1 mM EGTA

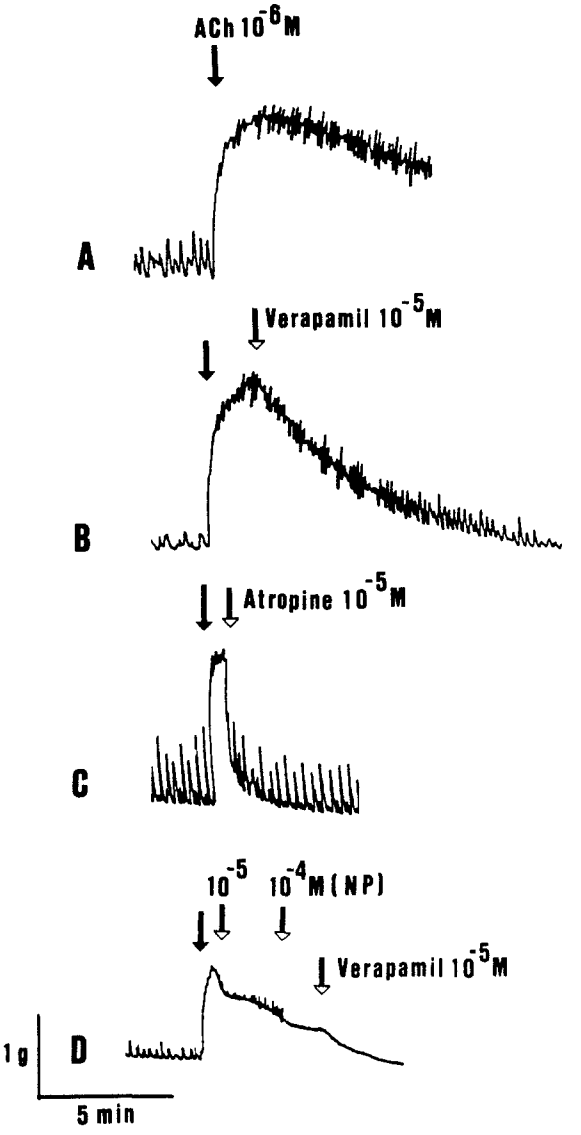


Fig. 4A–D. Relaxing effects of verapamil, atropine and sodium nitroprusside (*NP*) on detrusor muscle contraction induced by acetylcholine: (A) control (B) addition of verapamil (C) addition of atropine (D) addition of NP followed by verapamil

caused dose-dependent contraction of the strips. Pretreatment of the strips with verapamil significantly inhibited the acetylcholine-induced contraction in a dose-dependent manner (Fig. 1). On the other hand, NP ($10^{-7}$ to $10^{-5}$ M) failed to suppress the acetylcholine-induced contraction (Fig. 1).

In $Ca^{2+}$-free Krebs' solution containing 0.01 mM EGTA, the interactions of acetylcholine with $Ca^{2+}$ were demonstrated. In these experiments, the bath medium was changed to the $Ca^{2+}$-free solution after initial equilibration in the normal Krebs' solution. This resulted in a decreased basal tension and eliminated spontaneous contractile activity. In Fig. 2, the responses of the strips to 2.5 mM $Ca^{2+}$ replenishment in the $Ca^{2+}$-free Krebs' solution (control), in the presence of acetylcholine ($10^{-6}$ M), acetylcholine plus verapamil ($10^{-6}$ M) or acetylcholine plus NP ($10^{-6}$ M) were compared. The $Ca^{2+}$ replenishment to the $Ca^{2+}$-free medium caused a contracton of the strips. The $Ca^{2+}$ replenishment following to the pretreatment with acetylcholine elicited a significanlty enhanced contraction ($p < 0.01$), which was significantly inhibited in the presence of verapamil ($p < 0.005$) but not affected by NP (Fig. 2).

In $Ca^{2+}$-free Krebs' solution containing 0.1 mM EGTA, the cumulative addition of acetylcholine caused dose-dependent and slight degree of contraction of the strips, at the higher concentrations exceeding $10^{-6}$ M (Fig. 3). The amplitude of the maximum contraction induced by acetylcholine in the $Ca^{2+}$-free solution was nearly 5% of that in the normal Krebs' solution. These acetylcholine-induced contrations in the $Ca^{2+}$-free medium were completely abolished by pretreatment with atropine ($10^{-5}$ M), but not affected by pretreatment with verapamil nor NP ($10^{-5}$ M).

Representative tracings of the relaxation studies are demonstrated in Fig. 4. Verapamil or atropine ($10^{-5}$ M) completely relaxed the contraction once induced by acetylcholine ($10^{-6}$ M) to the basal tension level (Fig. 4B and C). NP ($10^{-5}$, $10^{-4}$ M) caused incomplete relaxation of the acetylcholine-induced contraction dose-dependently, and the following addition of verapamil ($10^{-5}$ M) made the relaxation complete (Fig. 4D).

## Discussion

Verapamil is one of the $Ca^{2+}$ antagonists referred as $Ca^{2+}$ entry blocker due to the ability to block selectively transmembrane $Ca^{2+}$ influx acting at the slow channels of the cell membrane [15]. Verapamil has been reported to inhibit selectively $Ca^{2+}$ influx at the concentrations lower than $10^{-4}$ M, although this drug might interfere with the availability of $Ca^{2+}$ at the intracellular site as well at higher concentrations exceeding $10^{-3}$ M [13]. In the present study, the pretreatment of the strips with verapamil inhibited the acetylcholine-induced contraction dose-dependently, and in the relaxation studies verapamil completely relaxed the contraction once induced by acetylcholine. In the experiments using the $Ca^{2+}$-free solution, verapamil inhibited the $Ca^{2+}$-induced contraction which was enhanced in the presence of acetylcholine. These results suggest that the acetylcholine-induced detrusor muscle contraction is mostly dependent of transmembrane extracellular $Ca^{2+}$ influx, in both its initiation and its maintenance.

Sodium nitroprusside (NP) has been known as a powerful vasodilating agent [10]. NP was demonstrated to act by a direct smooth muscle relaxing action, and specifically by interfering with intracellular $Ca^{2+}$ independent of $Ca^{2+}$ influx [7, 9]. Zsoter [16] also showed that NP did not alter the uptake of $^{45}Ca$ using the Lanthanum method in rabbit mesenteric artery and vein. The exact intracellular site of action of NP, however, remains to be elucidated. The efflux of $^{45}Ca$ was reported to be enhanced by NP [16]. Other possible modes of action of this drug were reported, including its effects on cellular rebinding of $Ca^{2+}$ or on cellular cyclic AMP and ATP levels [9]. NP has been reported to relax the smooth muscle of blood vessels [6, 7] uterus [6] and stomach [6]. However, there have been no data published on the effect of NP on the bladder detrusor muscle. In the present study, the pretreatment of the strips with NP failed to suppress the acetylcholine-induced contraction in the normal Krebs' solution and the $Ca^{2+}$-induced contraction in the $Ca^{2+}$-free solution containing acetylcholine. By contrast, NP showed an incomplete relaxation of the contraction once induced by acetylcholine. In the $Ca^{2+}$-free medium containing 0.1 mM EGTA, acetylcholine caused a dose-dependent slight contraction of the strips. This contraction was completely abolished by the pretreatment of the strips with atropine, suggesting that it was caused specifically by the action of acetylcholine mediated through cholinergic (muscarinic) receptors, independent of extracellular $Ca^{2+}$ influx. These results indicate that some intracellular $Ca^{2+}$ fractions at least partially participate in the acetylcholine-induced contraction. It is assumed that the intracellular $Ca^{2+}$ fractions might be operational in the maintenance of the contraction, although the effect of intracellular $Ca^{2+}$ seems to be less significant in comparison with the extracellular $Ca^{2+}$ influx and is probably not primarily responsible for initiating the contraction.

The present sutdy, however, could not demonstrate exactly the intracellular site of the action of acetylcholine on detrusor smooth muscle. Further, NP partially relaxed the acteylcholine-induced contraction, but could not inhibit the contraction induced by acetylcholine in the $Ca^{2+}$-free medium. We could not postulate a firm hypothesis to explain these results. These results probably imply that the effects of acetylcholine on the intracellular $Ca^{2+}$ fractions will rely upon multiple mechanisms.

Calcium antagonists which have the same action as verapamil include D-600 (methoxy-verapamil), nifedipine and prenylamine [15]. These $Ca^{2+}$ entry blockers have also been reported to inhibit the contraction of detrusor smooth muscle induced by acetylcholine [8], norepinephrine [8], electric stimulation [8], potassium [5, 8] and prostaglandin $F_2$ alpha [5]. The remarkable inhibitory effects of the $Ca^{2+}$ entry blockers on the detrusor muscle contractility are encouraging for possible clinical application of these drugs to treatment of hyperactive bladder disorders.

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M. Gotoh, M.D.
Department of Urology
Nagoya University
School of Medicine
65 Tsurumai-cho
Showa-ku
Nagoya
466 Japan