



Effect of pregnancy on the cholinergic responses of the bladder: role of acetylcholinesterase

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Abstract

Objectives Pregnancy is associated with many functional changes of the urinary bladder. It was reported that most of healthy women complain from urinary symptoms during pregnancy. The parasympathetic system is mainly mediating bladder emptying. The aim of the study is to investigate the cholinergic effect and the role of acetylcholinesterase in the bladder during pregnancy.

Methods Sixteen rats were used in the present study as control group (non-pregnant) and pregnant group (18–20 days pregnant). Isolated urinary smooth muscle strips were suspended in organ baths filled with Krebs' solution for isometric tension recording.

Results Electric field stimulation (EFS), (0.1–40 Hz), of the control and pregnant bladder preparations produced frequency-dependent contractions. Atropine (1 μ M) inhibited EFS-induced contractions in the two groups by 65% and 50% respectively indicating the response of cholinergic innervation. Neostigmine significantly enhanced EFS responses, confirming its selectivity for inhibiting acetylcholinesterase which is responsible for termination of acetylcholine. Concentration–response curves for acetylcholine were reduced in pregnant group than control. Concentration–response curves for ATP were increased in pregnant group than control. Neostigmine augmented concentration–response curves for acetylcholine in control and pregnant groups. The effect of neostigmine on acetylcholine contractile responses in pregnancy group was higher than in control.

Conclusions Urinary bladder dysfunction during pregnancy might be due to augmentation of acetylcholinesterase effect. This will lead to the decrease in response to cholinergic stimuli. New pharmaceutical drugs specifically affecting the enzyme in the bladder can help in avoiding the unpleasant urinary symptoms during pregnancy.

Keywords Bladder · Acetylcholinesterase · Pregnancy · Cholinergic innervation · Muscarinic receptors

Introduction

The lower urinary tract has two main functions: store urine without leakage for a suitable period and rapidly expel the urine during micturition. The urinary bladder smooth muscle has to relax and elongate during filling. Force generation and shortening must be initiated fast, be synchronized, and occur over a large length range during micturition. These relaxation and contraction activities require regulation. The autonomic nervous systems controlling the bladder function. The rat bladder receives a dual excitatory innervation;

acetylcholine (ACh) and adenosine triphosphate (ATP) are co-released from parasympathetic nerves [1, 2]. The parasympathetic system with the activation of muscarinic cholinergic receptors and purinergic receptors in various animal species are mediated bladder emptying, causing contraction of the detrusor muscle and relaxation of the sphincter [3, 4].

Acetylcholinesterase is an enzyme that cleaves acetylcholine to acetate and choline, so terminates its actions. It is located both pre- and post-synaptically in the nerve terminal where anticholinesterase can provoke its response. It has a role in regulating the free acetylcholine concentration. It is quite specific for acetylcholine and closely related esters. Acetylcholinesterase inhibitors prolonged the cholinergic action by preventing the degradation of acetylcholine. This leads to accumulation of acetylcholine in the synaptic space. Therefore, it can provoke a response at muscarinic receptors. Neostigmine is a synthetic

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compound, and it reversibly inhibits acetylcholinesterase leading to enhancement of acetylcholine activity. It is therapeutically used to contract the bladder [5].

Pregnancy is associated with functional changes of the urinary bladder. It was reported that most of healthy women have urinary symptoms during pregnancy [6]. Urinary frequency, increased residual volume and stress incontinence are the major complain [7]. It was reported that the response of the bladder to cholinergic stimulation was reduced due to decreased muscarinic receptor density during pregnancy [7–10]. Since cholinergic response is the main contributor for contraction, the aim of this study was to investigate the effect of cholinergic innervation, and the role of acetylcholinesterase in the reduction of cholinergic response in the bladder during pregnancy.

Materials and methods

Animals

Sixteen adult female Sprague–Dawley rats weighing approximately 200–250 g and matched by date of birth and were divided into two groups, control group (non-pregnant rats) and pregnant group (18–20 days pregnancy). The rats were housed on a 12 h light/dark cycle. The rats had free access to standard laboratory tap water and food and the room temperature was kept at 21 °C. The study was done in accordance with guidelines agreed by the Institutional Animal Care and Use Committee of Kuwait University.

Preparation of bladder strips

Each rat was sacrificed, and the urinary bladder was removed and placed in Petri dish containing Krebs' solution of the following composition (mM): NaCl 118, KCl 5.9, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 26, CaCl₂ 2, glucose 11.1, at pH 7.4. The bladder was cut longitudinally into two equal strips from control rats, and pregnant rats. They were suspended in 10 ml organ baths containing Krebs' solution, maintained at 37 °C and aerated with 95% O₂ and 5% CO₂. Isometric tension was recorded using a computerized, automated transducer system (Schuler organ bath type 809; Hugo Sachs Elektronik) which was connected to a Gould recorder. Each preparation was allowed to equilibrate under a resting tension of 1 g for 60 min, during which the bath fluid was changed twice. At the end of each experiment, the muscle was dried by a filter paper and weighed. Contractile responses were calculated as milligrams per milligram tissue weight or the responses were calculated as % to maximum response.

Electric field stimulation studies (EFS)

The bladder strips were passed through a pair of platinum ring electrodes to perform EFS experiments. The electrodes were connected to a Grass S8800 stimulator, delivering square wave pulses. 50 V, 0.5 ms for 10 s using frequency ranging from 0.1 to 40 Hz, were previously determined as optimum electrical stimulations parameters, were used in the present study. Frequency–response curves were done using the previous parameters every 3 min. Subsequently after resting period of 20 min, frequency–response curves were repeated in the presence of different drugs.

Drugs

Acetylcholine hydrochloride, neostigmine bromide, tetrodotoxin (TTX), adenosine triphosphate (ATP), and atropine sulfate were obtained from Sigma Chemicals, St. Louis, MO. All drugs were dissolved in distilled water.

Calculation

Data are presented as mean \pm SEM of (*n*) experiments. Differences between two mean values were compared using Student's *t* test paired. The difference was assumed to be significant at $P < 0.05$.

Results

Electric field stimulation-induced contraction

EFS (0.1–40 Hz) of the control and pregnant bladder preparations produced frequency-dependent contractions. The contractions were rapid in onset and stopped immediately when stimulation ceased. There is no difference between control and pregnant group in EFS responses as shown in Fig. 1a. These contractions were sensitive and abolished by TTX (1 μ M) confirming that they were neurogenically mediated. Incubation of the bladder strips for 30 min with atropine (1 μ M), inhibited EFS-induced contractions in the two groups by 65% and 50% respectively as shown in Fig. 1b, c. The inhibition of the control group was more than pregnant group indicating that cholinergic effect is more pronounced in the bladder of the control rat than during pregnancy. This result confirming that mainly the excitatory innervation of rat bladder smooth muscle is cholinergic in origin. EFS-induced contractions last 10 s, while in the presence of atropine they last 4 s. The peak of contraction reached after 10 s and the contraction

Fig. 1 a Frequency-induced contraction to electrical field stimulation (50 V, 0.5 ms, 10 s) of urinary bladder strips from (filled circle) control and (open circle) pregnant rats. Each point represents the mean \pm SEM of four rats. Significant difference between normal and pregnant groups ($*P < 0.05$). **b** Effect of atropine (1 μ M) on frequency-induced contraction to electrical field stimulation (50 V, 0.5 ms, 10 s) of urinary bladder strips (filled circle) control; and (open circle) after atropine. Each point represents the mean \pm SEM of four rats. Significant difference between normal and pregnant groups ($*P < 0.05$). **c** Effect of atropine (1 μ M) on frequency-induced contraction to electrical field stimulation (50 V, 0.5 ms, 10 s) of urinary bladder strips (filled circle) pregnant; and (open circle) after atropine. Each point represents the mean \pm SEM of four rats. Significant difference between normal and pregnant groups ($*P < 0.05$)

only stopped when the stimulation was ceased. No relaxant responses to EFS were observed in the presence of atropine.

Effect of neostigmine on EFS

Neostigmine, acetylcholinesterase inhibitor, (10 μ M) induced contraction of bladder strips. The incubation of the bladder strips with neostigmine resulted in significantly greater contractile EFS responses, confirming its selectivity for inhibiting acetylcholinesterase which is responsible for termination of acetylcholine as shown in Fig. 2.

Effect of cholinergic agonist on bladder strips

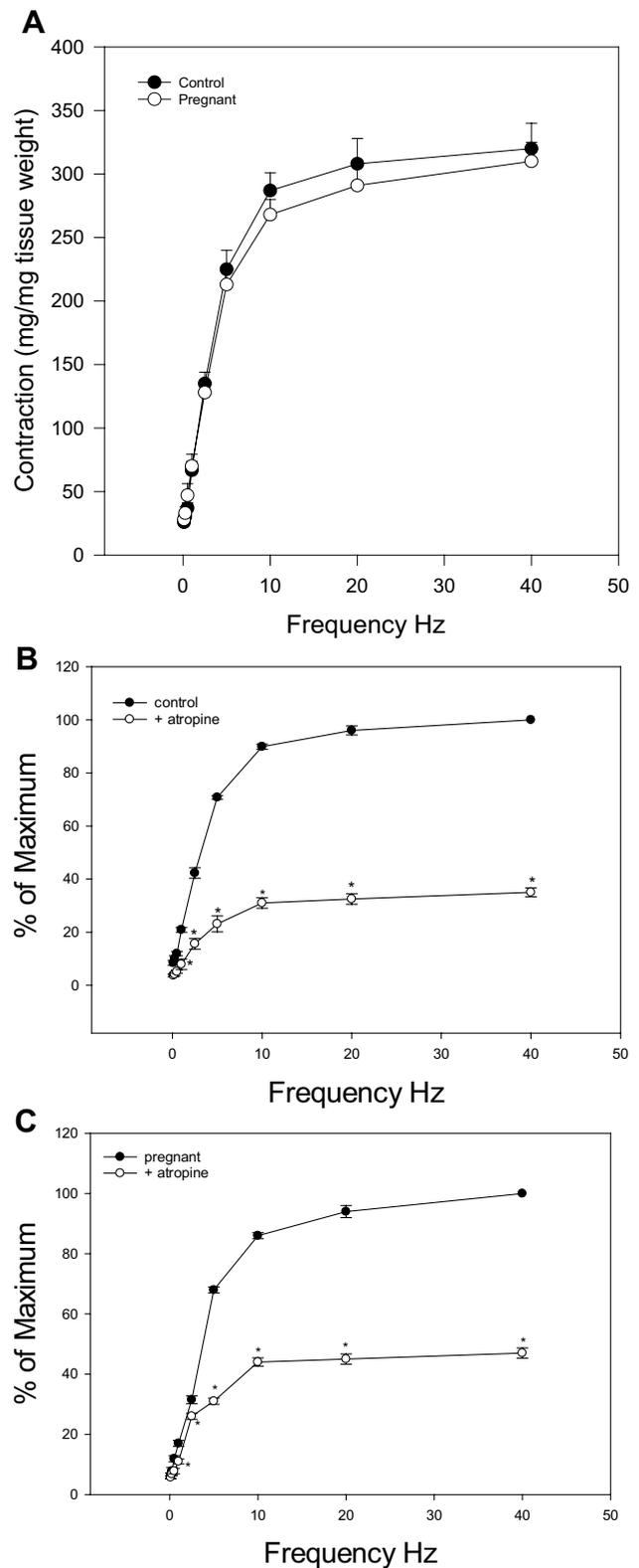
Acetylcholine (0.1 μ M–1 mM) induced concentration-dependent contractions of bladder strips as shown in Fig. 3. Dose–response curves for acetylcholine for control and pregnant groups were obtained. The effect was significantly inhibited in pregnant group than control.

Effect of ATP on bladder strips

ATP (0.1 μ M–1 mM) induced concentration-dependent contractions of bladder strips as shown in Fig. 4. Concentration–response curves for ATP for control and pregnant groups were obtained. The effect was significantly increased in pregnant group than control.

Effect of neostigmine on acetylcholine-induced contraction

Neostigmine (10 μ M) induced contraction of bladder strips. Pretreatment of the bladder strips with neostigmine augmented concentration–response curves for acetylcholine in control and pregnant groups as shown in Fig. 5a, b. The enhancement of neostigmine on acetylcholine contractile effects in pregnancy group was greater than that in control bladder strips. The results indicated that acetylcholinesterase was stronger during pregnancy than normal situations.



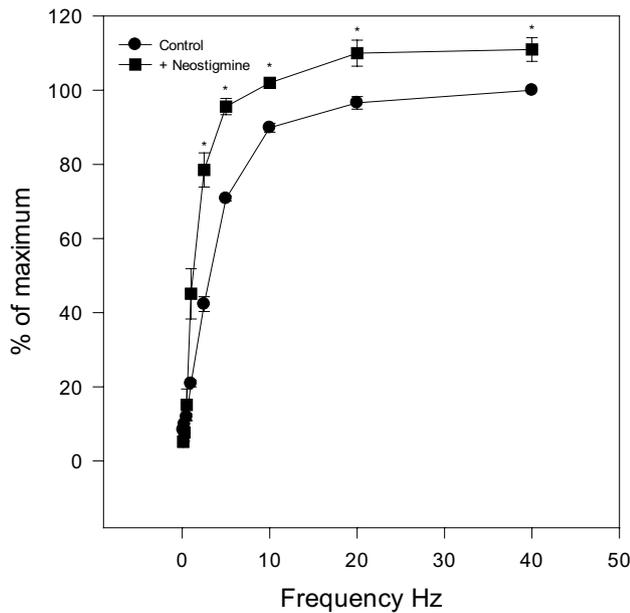


Fig. 2 Frequency-induced contraction to electrical field stimulation (50 V, 0.5 ms, 10 s) of urinary bladder strips (filled circle) control and (filled square) after neostigmine (10 μ M). Each point represents the mean \pm SEM of four rats. Significant difference between before and after neostigmine (* P < 0.05)

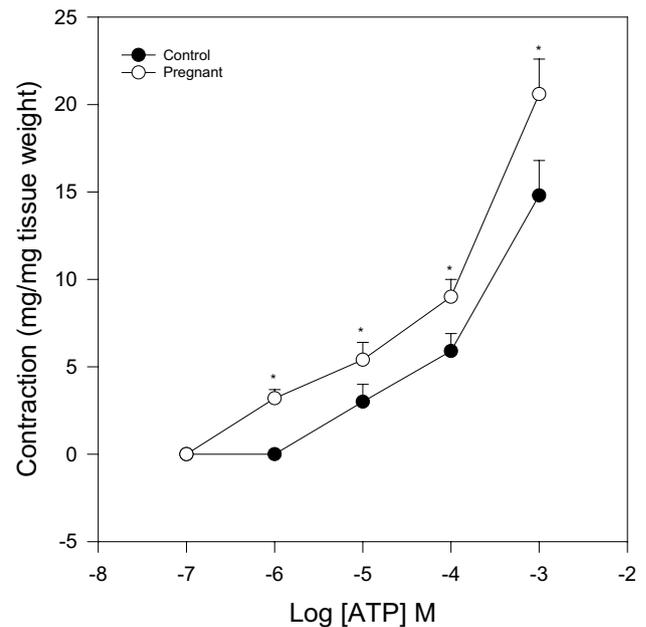


Fig. 4 ATP-induced contractions of urinary bladder (filled circle) control; and (open circle) pregnant rats. Each point represents the mean \pm SEM of four rats. Significant difference between normal and pregnant groups (* P < 0.05)

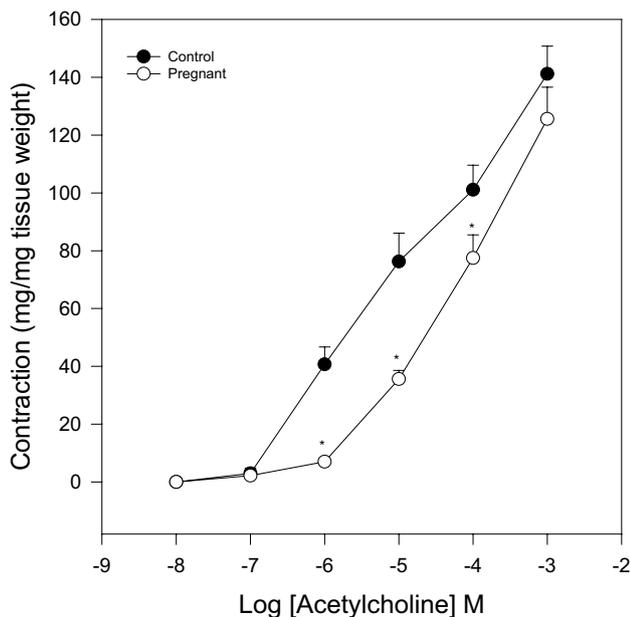


Fig. 3 Acetylcholine-induced contractions of urinary bladder (filled circle) control; and (open circle) pregnant rats. Each point represents the mean \pm SEM of four rats. Significant difference between normal and pregnant groups (* P < 0.05)

Discussion

The function of urinary bladder organ is to collect and store urine at low pressure, and then to expel the urine via a highly coordinated contraction. It is known that the control of bladder function is through the autonomic nervous system. The parasympathetic system is mediated bladder emptying with the activation of muscarinic cholinergic receptors and purinergic receptors [10]. During voiding the bladder, the parasympathetic system is eliciting contraction of the detrusor and relaxation of sphincter. Excitatory postganglionic parasympathetic neuromuscular transmission is a mixture of cholinergic and purinergic transmission, with acetylcholine and ATP, acting probably as co-transmitters. In the bladder, inhibitory transmission is predominantly postganglionic adrenergic innervation. Many other neurotransmitters have putative physiological roles not as neurotransmitters but as neuromodulators, potentiating or inhibiting adrenergic, cholinergic or purinergic activity [11], resulting in urine retention or overflow incontinence. Pregnancy alter different responses on urinary bladder [12]. The present study confirmed that cholinergic innervation is the major component in the contractile system in the rat urinary bladder. It is known that stimulation of muscarinic receptors raises intracellular inositol triphosphate (IP₃) levels and releases intracellular calcium in the initiation of contraction [13, 14]. The cholinergic innervation effect is more pronounced in the control group than in pregnant group. In addition, the effect

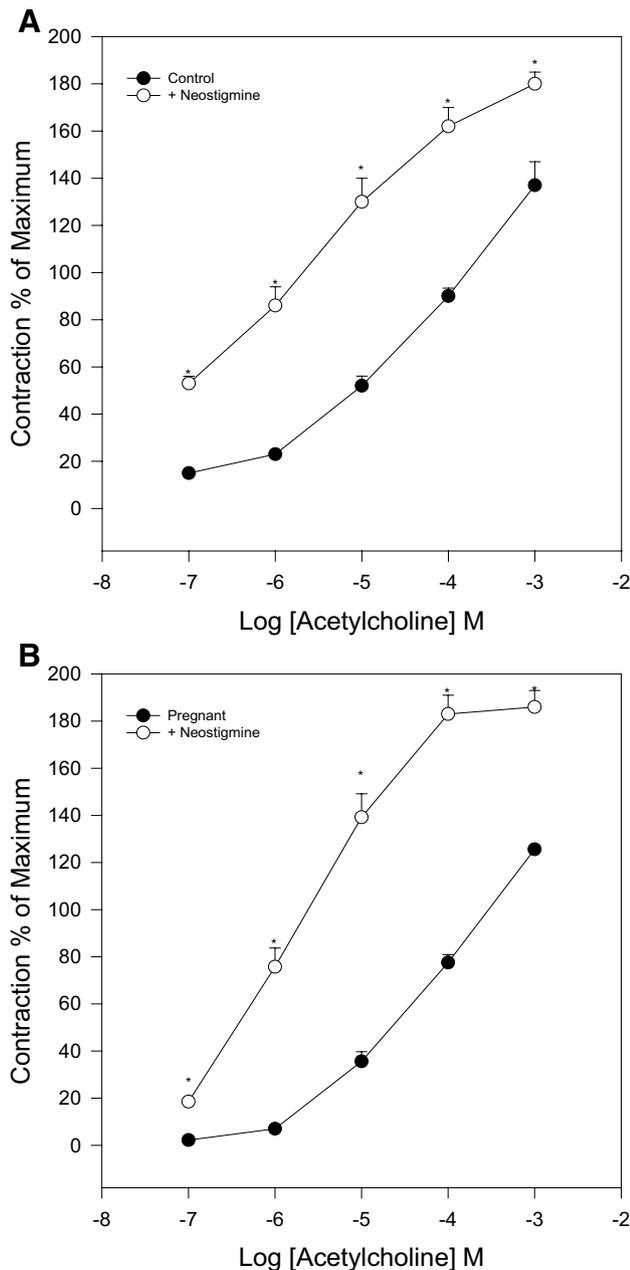


Fig. 5 **a** Effect of neostigmine (10 μ M) on acetylcholine concentration-contraction curves of rat urinary bladder strips (filled circle) control; and (open circle) after neostigmine. Each point represents the mean \pm SEM of four rats. Significant difference between before and after neostigmine ($*P < 0.05$). **b** Effect of neostigmine (10 μ M) on acetylcholine concentration-contraction curves of urinary bladder strips from (filled circle) pregnant; and (open circle) after neostigmine. Each point represents the mean \pm SEM of four rats. Significant difference between before and after neostigmine ($*P < 0.05$)

of exogenous acetylcholine was reduced during pregnancy. While exogenous ATP showed increase in its contractile response during pregnancy, which acts through P_2 receptors [15–17]. The bladder response to ATP is mediated by

activation of a ligand-gated cation channel (P_2X receptor) that leads the influx of extracellular Ca^{2+} , whereas uridine triphosphate and adenosine 5-*O*-(2-thiodiphosphate) might act through G protein-coupled receptors (P_2Y_2 or P_2Y_4) to cause smooth muscle contractions via a phospholipase C/ IP_3 signaling pathway [18] and release of intracellular Ca^{2+} . This study showed that there is no difference in the contractile response between control and pregnant groups due to EFS. But, this is not mean that the cholinergic responses are equal in control and pregnant groups. Atropine significantly inhibited the contractile response in control group than pregnant group. Exogenous acetylcholine confirmed the result. This indicates the decrease in endogenous cholinergic effect during pregnancy. While exogenous ATP evoked significant contractile effect in pregnant group than control, which can be considered a compensatory effect to the decrease in cholinergic effect. Acetylcholine is inactivated very rapidly within about 1 ms in the synaptic cleft by acetylcholinesterase. Neostigmine is a reversible acetylcholinesterase inhibitor, increases the time for terminations to minutes, and therefore it enhances the effect of acetylcholine. Acetylcholine induced contraction of isolated bladder which increased by cholinesterase inhibitors and abolished by atropine, which mediated by stimulation of muscarinic receptors. Neostigmine is used therapeutically to treat bladder under several situations [19, 20]. The present study showed that acetylcholinesterase inhibitors augmented the effect of cholinergic innervation. Acetylcholinesterase is more potent during pregnancy since neostigmine enhances the contractile effect of acetylcholine in pregnant group than control. This result is compatible with our previous study, Mustafa and Ismael [21], which proved that diabetes mellitus increased the presence or activity of cholinesterase in urinary bladder leading to urinary bladder problems. The increase of acetylcholinesterase effect during pregnancy seems to be transient and it gets back to normal after delivery. This can raise a question of the effect of hormonal changes during pregnancy and the enzyme.

We conclude that urinary bladder dysfunction during pregnancy might be due to augmentation of acetylcholinesterase' effect which will decrease the response to cholinergic stimuli. New specific drugs working on the enzyme can help avoiding the complain from the annoying urinary symptoms during pregnancy.

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Compliance with ethical standards

Conflict of interest The author declares that she has no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Research involving human participants There are no human participants included in the study.

References

- Burnstock G (2014) Purinergic signalling in the urinary tract in health and disease. *Purinergic Signal* 10(1):103–155
- Lawrence GW, Aoki R, Dolly O (2010) Excitatory cholinergic and purinergic signaling in bladder are equally susceptible to botulinum neurotoxin A consistent with co-release of transmitters from efferent fibers. *J Pharmacol Exp Ther* 334:1080–1086
- Levin RM, Longhurst PA, Kato K, Elbadawi A, Wein AJ, McGuire EJ (1990) Comparative physiology and pharmacology of the cat and rabbit urinary bladder. *J Urol* 143(4):848–852
- Igawa Y, Mattiasson A, Andersson KE (1993) Functional importance of cholinergic and purinergic neurotransmission for micturition contraction in the normal, unanaesthetized rat. *Br J Pharmacol* 109:473–479
- Ward TR, Ferris DJ, Tilson HA, Mundy WR (1993) Correlation of the anticholinesterase activity of a series of organophosphates with their ability to compete with agonist binding to muscarinic receptors. *Toxicol Appl Pharmacol* 122:300–307
- Chaliha C, Stanton SL (2002) Urological problems in pregnancy. *BJU Int* 89(5):469–476
- Tong YC, Hung YC, Lin JS, Hsu CT, Cheng JT (1995) Effects of pregnancy and progesterone on autonomic function in the rat urinary bladder. *Pharmacology* 50(3):192–200
- Elliott RA, Castleden CM (1994) Effect of progestogens and oestrogens on the contractile response of rat detrusor muscle to electrical field stimulation. *Clin Sci (London)* 87(3):337–342
- Knight GE, Burnstock G (2004) The effect of pregnancy and the oestrus cycle on purinergic and cholinergic responses of the rat urinary bladder. *Neuropharmacology* 46(7):1049–1056
- Levin RM, Zderic SA, Ewalt DH, Duckett JW, Wein AJ (1991) Effects of pregnancy on muscarinic receptor density and function in the rabbit urinary bladder. *Pharmacology* 43(2):69–77
- Hoyle CH (1994) Non-adrenergic, non-cholinergic control of the urinary bladder. *World J Urol* 12(5):233–244
- Mustafa S (2018) Effect of pregnancy on cooling tone and rhythmic contractions of the rat urinary bladder. *Int Urol Nephrol* 50:833–838
- Iacovou JW, Hill SJ, Birmingham AT (1990) Agonist-induced contraction and accumulation of inositol phosphates in the guinea-pig detrusor: evidence that muscarinic and purinergic receptors raise intracellular calcium by different mechanisms. *J Urol* 144(3):775–779
- Wuest M, Hiller N, Braeter M, Hakenberg OW, Wirth MP, Ravens U (2007) Contribution of Ca^{2+} influx to carbachol-induced detrusor contraction is different in human urinary bladder compared to pig and mouse. *Eur J Pharmacol* 22(1–3):180–189 565(
- Burnstock G, Kennedy C (1985) Is there a basis for distinguishing two types of P2-purinoceptor? *Gen Pharmacol* 16(5):433–440
- Abbracchio MP, Burnstock G (1994) Purinoceptors: are there families of P2X and P2Y purinoceptors? *Pharmacol Ther* 64(3):445–475
- Ralevic V, Burnstock G (1998) Receptors for purines and pyrimidines. *Pharmacol Rev* 50(3):413–492
- Burnstock G, Boeynaems JM (2014) Purinergic signalling and immune cells. *Purinergic Signal* 10(4):529–564
- Mokrý J, Jakubsov M, Svihra J, Urdzik J, Hudec M, Nosalova G, Kliment J (2005) Reactivity of urinary bladder smooth muscle in guinea pigs to acetylcholine and carbachol: participation of acetylcholinesterase. *Physiol Res* 54:453–458
- Stallard S, Prescott S (1988) Postoperative urinary retention in general surgical patients. *Br J Surg* 75(11):1141–1143
- Mustafa S (2014) Reactivity of diabetic urinary bladder to the cholinesterase inhibitor neostigmine. *Urology* 84(6):1549.e1–1549.e5