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# **ATP transients accompany spontaneous contractions in isolated guinea-pig detrusor smooth muscle**

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*What is the central question of this study?*

Overactive bladder (OAB) is associated with enhanced spontaneous contractions, but their origins are unclear. This study aimed to characterise accompanying ATP transients.

*What is the main finding and its importance?*

Spontaneous detrusor contractions were accompanied by transient increases of ATP and their appearance was delayed by previous activation of efferent nerves to detrusor. This indicates spontaneous ATP release from nerve terminals supports spontaneous contractions. ATP is a functional excitatory neurotransmitter in human bladder only in pathologies such as OAB. A potential drug target is revealed to manage this condition.

## Abstract

Spontaneous contractions are characteristic of the bladder wall, but their origins remain unclear. Activity is reduced if the mucosa is removed but does not disappear, suggesting a fraction arises from the detrusor. We tested the hypothesis that spontaneous detrusor contractions arise from spontaneous ATP release. Guinea-pig detrusor strips, without mucosa, were superfused with Tyrode's solution at 36°C. Preparations were electrically field stimulated (EFS, 3-sec trains at 90-second intervals) to produce nerve-mediated contractions, abolished by 1  $\mu$ M tetrodotoxin. Amperometric ATP electrodes on the preparation surface recorded any released ATP. Spontaneous contractions and ATP transients were recorded between EFS stimulation. Nerve-mediated contractions were attenuated by atropine and  $\alpha,\beta$  methylene ATP: in combination they nearly abolished contractions, as did nifedipine. Contractions were accompanied by ATP transients that were unaffected by atropine but inhibited by TTX and greatly attenuated by nifedipine. Spontaneous contractions were accompanied by ATP transients with a close correlation between the magnitudes of both transients. ATP and contractile transients persisted with TTX, atropine and nifedipine. Immediately after a nerve-mediated contraction and ATP transient there was a longer interval than normal before spontaneous activity resumed. Spontaneous contractions and ATP transients are proposed to arise from ATP leakage from nerve terminals innervating the detrusor. Extracellular ATP has a greater functional significance in humans who suffer from detrusor overactivity (spontaneous bladder contractions associated with incontinence) due to its reduced hydrolysis at the nerve-muscle interface. This study shows the origin of spontaneous activity that may be exploited to develop a therapeutic management of this condition.

## Introduction

Spontaneous contractions are a feature of the bladder; from the whole organ, through to bladder sheets, isolated strips and myocytes. However, it is unlikely that they have a single origin, as their frequency can vary greatly between different preparations and more significantly removal of the mucosa greatly suppresses, but does not completely abolish, them (Kushida & Fry, 2016). Whole bladder and bladder sheets optical imaging experiments provide compelling evidence that spontaneous activity can arise from the mucosa and then propagates to the detrusor (Kanai et al., 2007). This spontaneous activity is enhanced by purinergic (P2Y) agonists (Fry et al., 2012). It is hypothesised that interstitial cells provide the medium for conduction, as they generate excitatory electrical responses in response to P2Y agonists and their number increases in conditions, such as spinal cord injury, when spontaneous activity is greatly enhanced. However, spontaneous activity persists when the mucosa is removed from isolated bladder wall samples, albeit at a lower level, and thus may also arise from the detrusor itself (Kushida & Fry, 2016).

Spontaneous contractions may result from basal electrical activity in parasympathetic nerves; from pacemaking activity in interstitial cells or by intrinsic myogenic activity. Spontaneous contractions are resistant to neurotoxins such as tetrodotoxin (TTX) but that does not exclude leakage of ATP or acetylcholine from nerve endings, and indeed this has been observed for both transmitters (Young et al., 2008; Zagorodnyuk et al., 2009). It is unlikely that interstitial cells (ICs) fulfil a pacemaking role in detrusor, as they do in the GI-tract, as spontaneous  $\text{Ca}^{2+}$  transients in neighbouring ICs and detrusor myocytes are not synchronised, with ICs showing a low asynchronous rate when compared to myocytes (Hashitani et al., 2004). However, interactions between detrusor myocytes and ICs have been proposed, for example as part of an afferent signalling pathway, involving a role for small conductance (SK)  $\text{K}^+$  channels (Heppner et al., 2016). Detrusor myocytes do develop spontaneous action potentials that are dependent on an interplay between  $\text{Ca}^{2+}$  and  $\text{K}^+$  channels (Hashitani &

Brading, 2003; Petkov, 2104). However, it remains unclear the extent to which they propagate in the detrusor syncytium as gap junctions are formed from sparse, low-conductance Cx45 channels (Sui et al., 2003), although computational studies are exploring the active cable properties of detrusor (Appukuttan et al 2018).

This study started from anecdotal observations by us and others (see Sibley, 1984) that spontaneous contractile activity often starts and persists when electrically-driven contractions are halted. We recorded their characteristics, pharmacological properties and their relationship to extracellular ATP transients also observed in these preparations.

## Methods

*Ethical Approval.* Procedures were approved by the Animal Welfare and Ethics Review Board of the University of Bristol (17/09/2014), in accordance with UK legislation (Animals (Scientific Procedures) Act 1986 (ASPA). The study also complies with ethical principles under which *Experimental Physiology* operates.

*Preparations and solutions.* Male Dunkin-Hartley guinea-pigs (<250 g) were sourced via the animal services unit, housed at 22°C with a 12hr light-dark cycle and with water and food available *ad libitum*. Animals were killed by injection with Na pentobarbital (200 mg.kg<sup>-1</sup>, i.p.) and cervical dislocation, verified by a lack of corneal and spinal reflexes, and the bladder immediately removed through a laparotomy. Strips of detrusor (5 mm length, 0.3-0.5 mm radius) were dissected and attached to a fixed hook and isometric force transducer in a horizontal trough. Preparations were superfused at 37°C with Tyrode's solution containing (mM): NaCl, 118; NaHCO<sub>3</sub>, 24; KCl, 4.0; NaH<sub>2</sub>PO<sub>4</sub>, 0.4; MgCl<sub>2</sub>, 1.0; CaCl<sub>2</sub>, 1.8; glucose, 6.1; Na pyruvate, 5.0; 5%CO<sub>2</sub>, 95%O<sub>2</sub>, pH 7.4. Nerve-mediated contractions generated by electrical field stimulation (EFS; 0.1 ms pulses, 1-40 Hz, 3-s train every 90-s) that were inhibited by tetrodotoxin (TTX, 1 µM). TTX, atropine, α,β-methylene ATP (ABMA) or nifedipine were added to the superfusate from aqueous stock solutions to the desired concentrations.

*ATP electrodes.* Amperometric ATP electrodes (Sarissa Biomedical Ltd, Coventry, UK) were used to measure nerve-mediated and spontaneous ATP release. Electrodes had an active tip of 2 mm length and 50 µm diameter and were placed on the surface of the preparation parallel to the longitudinal axis. A null electrode, lacking the sensing surface was similarly placed and both polarised to 0.65 V. Electrode outputs formed the inputs to a home-made differential amplifier, with a very high common mode rejection that removed artefacts from field stimulation electrodes, and the output digitised and recorded. Glycerol (2 mM) was added to Tyrode's superfusate as it is required for the

enzymatic detection of ATP. Prior to experiments the system was calibrated by exposure to 10  $\mu\text{M}$   $\text{Na}_2\text{ATP}$  – electrodes had a linear response between 0.2 and 50  $\mu\text{M}$  ATP.

*Data presentation and analysis.* Data are shown as mean $\pm$ SD, derived from  $n$  observations from  $N$  separate preparations. For nerve-mediated tension and ATP responses peak amplitude values were recorded and force-frequency relationships fitted to:

$$S = (S_{\max}f^n)/(f^n + f_{1/2}^n) \quad 1)$$

where  $S_{\max}$  is the maximum estimated response at high frequencies,  $f_{1/2}$  the frequency to elicit  $S_{\max}/2$  and  $n$  is a constant. Curve-fitting was by a non-linear iterative fit program (KaleidaGraph, Synergy Software, CA, USA). Peak values of spontaneous force and ATP transients were also recorded and correlation tested by Spearman's rank correlation. The slope of the relationship was derived from a linear least-squares fit. Differences between data sets were tested by Student's  $t$ -test; the null hypothesis was rejected at  $p < 0.05$ .



## Results

*Tension and ATP transients in response to electrical field stimulation (EFS).* Contractions were accompanied by transient increases of ATP over the range of stimulation frequencies from 1-32 Hz. At lower frequencies (1-4 Hz) the profiles of tension and ATP transients were similar (figure 1A), at higher frequencies there was often a prolonged decay phase (figure 1B). ATP transients were not routinely recorded above 16 Hz stimulation, as they did not change in amplitude at higher frequencies. In addition, the peak of the ATP transient was generally delayed compared to the contraction: the mean delay was  $1.49 \pm 1.00$  s,  $n=62$  transients,  $N=10$  preparations). Contraction and ATP transient amplitudes were frequency-dependent (figure 1C,D) although there were some differences in the frequency profile and the response to interventions. In control, the tension  $f_{1/2}$  frequency was significantly greater than the corresponding ATP value ( $7.6 \pm 4.3$  vs  $3.3 \pm 2.1$  Hz,  $N=8$ ).

### Figure 1 near here

With atropine (1  $\mu$ M), estimated  $S_{\max}$  for tension recordings was reduced to  $35.6 \pm 5.9\%$  of control whereas there was no significant effect on the ATP transients  $85.6 \pm 12.8\%$  of control ( $N=6$ ). The  $f_{1/2}$  frequency for tension recordings was also significantly reduced compared to control ( $4.7 \pm 3.1$  Hz,  $N=6$ ) and not significantly different from that for ATP transients. Both contractions and ATP transients were completely abolished by tetrodotoxin (1  $\mu$ M,  $N=6$ ) and greatly attenuated by nifedipine (1  $\mu$ M,  $N=5$ ):  $S_{\max}$  for contractions to  $3.3 \pm 0.9\%$  of control;  $S_{\max}$  for ATP transients recordings to  $12.6 \pm 1.5\%$  of control (figure 1C,D,  $N=6$ ). Recording ATP transients was not possible in the presence of ABMA (1  $\mu$ M) as the latter desensitised the electrodes, but  $S_{\max}$  for tension recordings was reduced to  $83.5 \pm 6.0\%$  of control and  $f_{1/2}$  frequency significantly increased compared to control ( $12.8 \pm 2.8$  Hz,  $N=5$ ).

*Spontaneous contractions and ATP transients.* Spontaneous contractions were recorded in eight of 11 preparations, and in six of these preparations ATP transients were simultaneously recorded, with a frequency of  $4.72 \pm 0.54 \text{ min}^{-1}$  ( $N=6$ , Figure 2A). The peak amplitudes of tension and ATP transients were normalised as percentages of corresponding values evoked at 4 Hz stimulation in the same preparation. In control conditions, the values for tension,  $T_{sp}$ , and ATP,  $ATP_{sp}$ , spontaneous transients were  $3.8 \pm 1.0\%$  and  $6.7 \pm 2.1\%$ , respectively ( $N=6$ ) compared to nerve-mediated transients elicited at 4 Hz stimulation. The ATP transient was also consistently delayed compared to the tension response (Figure 2A, inset) by  $2.51 \pm 0.96 \text{ s}$  ( $n=100$ ,  $N=6$ ), similar to those evoked by nerve-mediated stimulation (figure 1A,B). Spontaneous tension and ATP transients were not significantly different from control in the presence of  $1 \mu\text{M}$  TTX ( $T_{sp}$   $3.0 \pm 1.1\%$  and  $ATP_{sp}$   $7.1 \pm 2.8\%$ ,  $N=5$ ) or atropine ( $T_{sp}$   $3.7 \pm 0.8\%$  and  $ATP_{sp}$   $8.4 \pm 2.2\%$ ,  $N=5$ ). With  $1 \mu\text{M}$  nifedipine  $T_{sp}$  was significantly ( $p < 0.05$ ) reduced but not abolished,  $ATP_{sp}$  was not significantly affected ( $T_{sp}$   $2.3 \pm 1.5\%$  and  $ATP_{sp}$   $5.2 \pm 1.7\%$ ,  $N=5$ ). In the case of TTX and nifedipine concentrations used respectively abolished or severely attenuated contractile responses evoked by electrical field stimulation (figure 1C). In addition, tension transients were also recorded in the presence of the non-hydrolysable ATP analogue,  $\alpha, \beta$ -methylene-ATP (ABMA), although ATP transients were not – see above. In the examples shown in figure 2B,C multiple peaks to the ATP and tension transients were observed that suggests that they may have arisen from different parts of the preparation. It is possible that the ATP transient originates in release of the nucleotide from muscle tissues due to the actual contraction of the tissue. This is unlikely (figure 2E), as there was a minimal response from the ATP electrode during a near maximum contracture generated by  $1 \mu\text{M}$  carbachol. Just prior to the contracture a small spontaneous contraction and accompanying ATP transient (arrowed) are seen to demonstrate the difference in the respective contractile responses.

**Figure 2 near here**

The magnitudes of the tension and ATP transients were highly correlated within a preparation and four sets of data from different preparations are shown: one in control solution and three others in the presence of TTX, nifedipine or atropine (figure 3A). The slopes of these relationships ( $\Delta T/\Delta ATP$ ) varied even during control ( $n=7, N=6$ ) and intervention (each  $n=5, N=5$ ) periods in the same preparation when separated by more than 30 minutes: overall 22 periods were analysed, each contributed between 14 and 53 pairs of transients, with slopes varying between 0.02 and 1.13 (figure 3B).

**Figure 3 near here**

The intervals between spontaneous transients was greater immediately after larger nerve-mediated ATP transients were generated (figure 4); this was a consistent feature under control conditions, when nerve-mediated responses were much greater. Thus, increasing the stimulation frequency generated successively large magnitude ATP transients, expressed as a percentage of that obtained at 4 Hz stimulation. As the stimulated transient increased in magnitude from 1 Hz through to 8 Hz, the interval to when the next spontaneous transient also increased. This phenomenon was observed in six preparations.

**Figure 4 near here**

## Discussion

*Spontaneous ATP and tension signals.* This study showed that spontaneous tension transients, in isolated detrusor smooth muscle, with the overlying mucosa removed, are accompanied by similar ATP transients. Contraction transients were smaller and less frequent than those generated when the mucosa was intact, where they arise from mucosal ATP release (Kushida & Fry, 2016). The correlation between tension and ATP transient magnitude was very significant suggesting a causal relationship between a transient increase of ATP to generate a spontaneous contraction. Before considering the origin of the ATP signal and its pharmacological properties two quantitative confounders must be considered. Firstly, the ATP signal was often delayed in comparison to the tension transient (figure 1A, 2A, but contrast figure 1B); counter-intuitive to a causal relationship. Secondly, the relationship between the ATP and tension recordings, the  $\Delta T/\Delta ATP$  ratio - varied more than 10-fold in different preparations (figure 3B). Both may be explained by a mathematical model of ATP diffusion from the preparation to an adjacent ATP electrode (below). This model shows that ATP diffusion imparts considerable temporal delays and attenuation of an electrode response, even at small distances from the preparation relative to its radius. This will explain the delay in the ATP transient and also the variable  $\Delta T/\Delta ATP$  ratio. Amperometric electrodes will thus generate noise-free, real-time qualitative data; however, comparison of quantitative data between preparations, when relative electrode positions will inevitably vary, requires more careful interpretation.

*A model of ATP diffusion from a muscle preparation to an ATP electrode.* The model was devised to test if the recorded delay of the ATP transient, compared to the tension transient, could in part be explained by diffusion time of ATP from the detrusor strip to the ATP electrode. The model (figure 5) is a radially symmetrical cylindrical preparation of radius  $b$ , and an ATP electrode external to the preparation at a mean distance,  $a$ , recording ATP concentration,  $c$ . The diffusion constant for ATP is  $D_1$  inside the preparation and  $D_2$  outside; the dimensionless parameter,  $\lambda = b/a$ .

Figure 5 near here

In cylindrical co-ordinates, the diffusion equation is:

$$\begin{aligned}\frac{\partial c}{\partial t} &= D_1 \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial c}{\partial r} \right), & r \leq a \\ \frac{\partial c}{\partial t} &= D_2 \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial c}{\partial r} \right), & r > a\end{aligned}\quad 2)$$

For continuous fluxes across  $r=a$

$$D_1 \frac{\partial c}{\partial r} \Big|_{r=a^-} = D_2 \frac{\partial c}{\partial r} \Big|_{r=a^+} \quad 3)$$

Assume  $D_1 = D_2 = D$  and define the Fourier-Bessel transform

$$\begin{aligned}\hat{c}(k, t) &= \int_0^\infty r c(r, t) J_0(kr) dr \\ c(r, t) &= \int_0^\infty k \hat{c}(k, t) J_0(kr) dk\end{aligned}\quad 4)$$

Thus,

$$\begin{aligned}\hat{c}(k, 0) &= \frac{c_0 b}{k} J_1(kb) \\ \hat{c}_t(k, t) &= -k^2 D \hat{c}(k, t)\end{aligned}\quad 5)$$

with solution

$$\hat{c}(k, t) = \frac{c_0 b}{k} J_1(kb) \cdot e^{-k^2 D t} \quad 6)$$

Inversion of the transform yields

$$c(r, t) = c_0 b \int_0^\infty J_1(kb) J_0(kr) \cdot e^{-k^2 D t} dk \quad 7)$$

Where  $J_0$  and  $J_1$  are Bessel functions of the first kind, of zeroth and first order respectively.

Rescale:  $r = Ra$ ,  $\tau = Dt/a^2$ , and make the integral substitution:  $u = ka$ . Then:

$$\tilde{c}(R, \tau) \equiv c \left( Ra, \frac{a^2 \tau}{D} \right) = c_0 \lambda \int_0^\infty J_1(\lambda u) J_0(Ru) e^{-\tau u^2} du \quad 8)$$

Equation 8) was evaluated in MATLAB for a concentration profile as a function of  $\tau$  for several values of  $\lambda$  from 0.95 to 0.1 (figure 6A). Outside the preparation  $c=0$  at  $t=0$ , but then increases to a transient maximum. As  $\lambda$  is reduced, i.e. the electrode is further from the preparation, the peak of the ATP profile is more delayed and attenuated. Thus, the delay in the ATP electrode response can

be attributed, at least in part, to a diffusion delay in ATP reaching the electrode. The abscissa may be converted to a real-time axis by multiplying  $\tau$  by  $a^2/D$ , figure 6A. At 25°C the ATP diffusion coefficient,  $D_{25}$ , in tissue or solutions of ionic strength similar to Tyrode's is  $\approx 4 \cdot 10^{-6} \text{ cm}^2 \cdot \text{s}^{-1}$  (Bowen & Martin, 1964; Hubley et al., 1995). At 37°C,  $D_{37}$  is calculated, assuming a  $Q_{10}$  value of 2.0, from:  $D_{37} = D_{25} \cdot 2^{(37-25/10)} \approx 9.2 \cdot 10^{-6} \text{ cm}^2 \cdot \text{s}^{-1}$ . For a preparation of radius,  $a=0.04 \text{ cm}$ ,  $a^2/D_{37} \approx 174 \text{ s}$ . Figure 6B plots the delay to reach peak ATP concentration as a function of  $\lambda$ ; values of several seconds, similar to those recorded experimentally, were achieved at  $\lambda=0.95$  or greater showing that close apposition of the electrode to the preparation still imparted significant delays.

**Figure 6 near here**

*The origin and pharmacology of the spontaneous ATP and tension transients.* The pharmacology of ATP and tension spontaneous transients is different from nerve-mediated responses. The neurotoxin TTX abolished nerve-mediated contractions and accompanying ATP transients, but did not significantly affect spontaneous counterparts. Nerve-mediated ATP release plays a more significant role in contractile generation at lower frequencies of stimulation (up to about 16 Hz). The continuing increase of force at greater frequencies, although ATP release plateaus, is consistent with a more important role for acetylcholine, as atropine had a greater action over this range. It is possible that ATP leaks from muscle cells during a spontaneous contraction that may arise for a separate reason. However, application of carbachol generated a large contraction but no ATP electrode output (data not shown). One possibility is transmitter leakage from nerve terminals, independent of nervous activity. Consistent with this hypothesis is that after a large ATP release from nerve terminals by electrical stimulation there was a longer-than-normal delay before the appearance of the next spontaneous ATP and tension transient (figure 4), this occurred in all preparations. Spontaneous acetylcholine and ATP release have been inferred in isolated guinea-pig detrusor as spontaneous contractions were blocked, respectively, by the antimuscarinic hyoscine

(Zagorodnyuk et al., 2009) and by purinergic (P2X<sub>1</sub>) receptor antagonists (Young et al., 2008). There was, however, no evidence of spontaneous ACh release under the conditions of these experiments as atropine had no effect on the magnitude of spontaneous contractions or ATP transients - see also Sibley (1984).

This study provides direct evidence of spontaneous ATP release in guinea-pig detrusor and is consistent with measurements of spontaneous excitatory potentials in detrusor preparations (Young et al., 2008). ATP transients could not be recorded in the presence of the non-hydrolysable ATP analogue,  $\alpha,\beta$ -methylene-ATP (ABMA), but spontaneous tension transients were not abolished. ABMA is an agonist to P2X<sub>1/3</sub> receptors, but prolonged exposure then desensitises them and the persistence of tension transients implies that any spontaneously released ATP does not act exclusively via this subtype. However, about 6% of the contraction arising from nerve-mediated ATP release has also been reported to be independent of P2X<sub>1/3</sub> receptor activation (Kennedy et al., 2007, Kennedy, 2015). This fraction is similar to the magnitude of the spontaneous tension transients reported here and it may be proposed that spontaneous ATP release targets this alternative signalling pathway. Moreover, spontaneous contractions were not completely abolished by nifedipine at a concentration that largely abolished nerve-mediated contractions, also suggesting an alternative pathway for contractile activation. The persistence of some contractile response with nifedipine is different from reports in the literature using similar mucosa-free preparations (Hashitani et al, 2004), but in this case spontaneous contractions were accompanied by action potentials, suggesting Ca<sup>2+</sup> influx through L-type Ca<sup>2+</sup> channels.

Spontaneous ATP release was independent of the presence of TTX. This suggests that if the origin is neural it does not occur from low base-line action potential generation in parasympathetic motor nerves. Spontaneous transmitter release is widely reported in the central nervous system and is via

presynaptic pathways different from those that regulate evoked release and may play a role in maintaining synaptic efficacy (Kavalali, 2015). In particular, nifedipine has an additional property of enhancing neurotransmitter release (Piriz et al, 2003) and may explain why the  $\text{Ca}^{2+}$ -antagonist had no effect on the magnitude of spontaneous contractions.

The observation that spontaneous contractile activity in isolated detrusor smooth muscle is accompanied by ATP release implies a mechanism that may add basal contractile tone so that upon nerve-mediated activation the muscle is not contracting from a flaccid state. Of interest will be to determine if this process is upregulated in condition associated with overactive bladder syndromes. Certainly, the magnitude of spontaneous contractions, their activation of greater regions of the bladder wall, and an increase of concomitant electrical and  $\text{Ca}^{2+}$  wave propagation is observed in bladder overactivity associated with spinal cord injury (Fry et al, 2012) The mechanisms described here may provide a basis for these phenomena and also highlight targets that may diminish this activity.

**Conclusions.** Detrusor smooth muscle exhibits spontaneous tension and accompanying ATP contractions, that are resistant in part to tetrodotoxin, nifedipine and atropine. The association between magnitudes of tension and ATP transients was very significant suggesting a causal relationship. We propose that spontaneous transients represent transmitter leakage from nerves in detrusor bundles.



## References

- Appukuttan, S., Padmakumar, M., Young, J.S., Brain, K.L. & Manchanda, R. (2018). Investigation of the syncytial nature of detrusor smooth muscle as a determinant of action potential shape. *Frontiers in Physiology* **9**: 1300. doi: 10.3389/fphys.2018.01300
- Fry, C.H., Young, J.S., Jabr, R.I., McCarthy, C., Ikeda, Y. & Kanai, A.J. (2012). Modulation of spontaneous activity in the overactive bladder: the role of P2Y agonists. *American Journal of Physiology, Renal Physiology* **302**: F1447-F1454. doi: 10.1152/ajprenal.00436.2011
- Hashitani, H. & Brading, A.F. (2003). Ionic basis for the regulation of spontaneous excitation in detrusor smooth muscle cells of the guinea-pig urinary bladder. *British Journal of Pharmacology* **140**: 159–169. doi: 10.1038/sj.bjp.0705320.
- Hashitani, H., Brading, A.F. & Suzuki, H. (2004). Correlation between spontaneous electrical, calcium and mechanical activity in detrusor smooth muscle of the guinea-pig bladder. *British Journal of Pharmacology* **141**: 183-193. doi: 10.1038/sj.bjp.0705602.
- Hashitani, H., Yanai, Y. & Suzuki, H. (2004). Role of interstitial cells and gap junctions in the transmission of spontaneous Ca<sup>2+</sup> signals in detrusor smooth muscles of the guinea-pig urinary bladder. *Journal of Physiology* **559**: 567–581. doi: 10.1113/jphysiol.2004.065136.
- Heppner, T.J., Tykocki, N.R., Hill-Eubanks, D. & Nelson, M.T. (2016). Transient contractions of urinary bladder smooth muscle are drivers of afferent nerve activity during filling. *Journal of Physiology* **147**: 323–335. doi: 10.1085/jgp.201511550
- Kanai, A., Roppolo, J., Ikeda, Y., Zabbarova, I., Tai, C., Birder, L., .... Fry, C.H. (2007). Origin of spontaneous activity in neonatal and adult rat bladders and its enhancement by stretch and muscarinic agonists. *American Journal of Physiology, Renal Physiology* **292**: F1065-1072. doi: 10.1152/ajprenal.00229.2006
- Kavalali, E.T. (2015). The mechanisms and functions of spontaneous neurotransmitter release. *Nature, Reviews in Neuroscience* **16**: 5-16. doi: 10.1038/nrn3875
- Kennedy, C., Tasker, P.N., Gallacher, G. & Westfall, T.D. (2007). Identification of atropine- and P2X<sub>1</sub> receptor antagonist-resistant, neurogenic contractions of the urinary bladder. *Journal of Neuroscience* **27**: 845-851. doi: 10.1523/JNEUROSCI.3115-06.2007
- Kennedy, C. (2015). ATP as a co-transmitter in the autonomic nervous system. *Autonomic Neuroscience* **191**; 2-15. doi: 10.1016/j.autneu.2015.04.004
- Kushida, N. & Fry, C.H. (2016). On the origin of spontaneous activity in the bladder. *BJU International* **117**: 982-992. doi: 10.1111/bju.13240

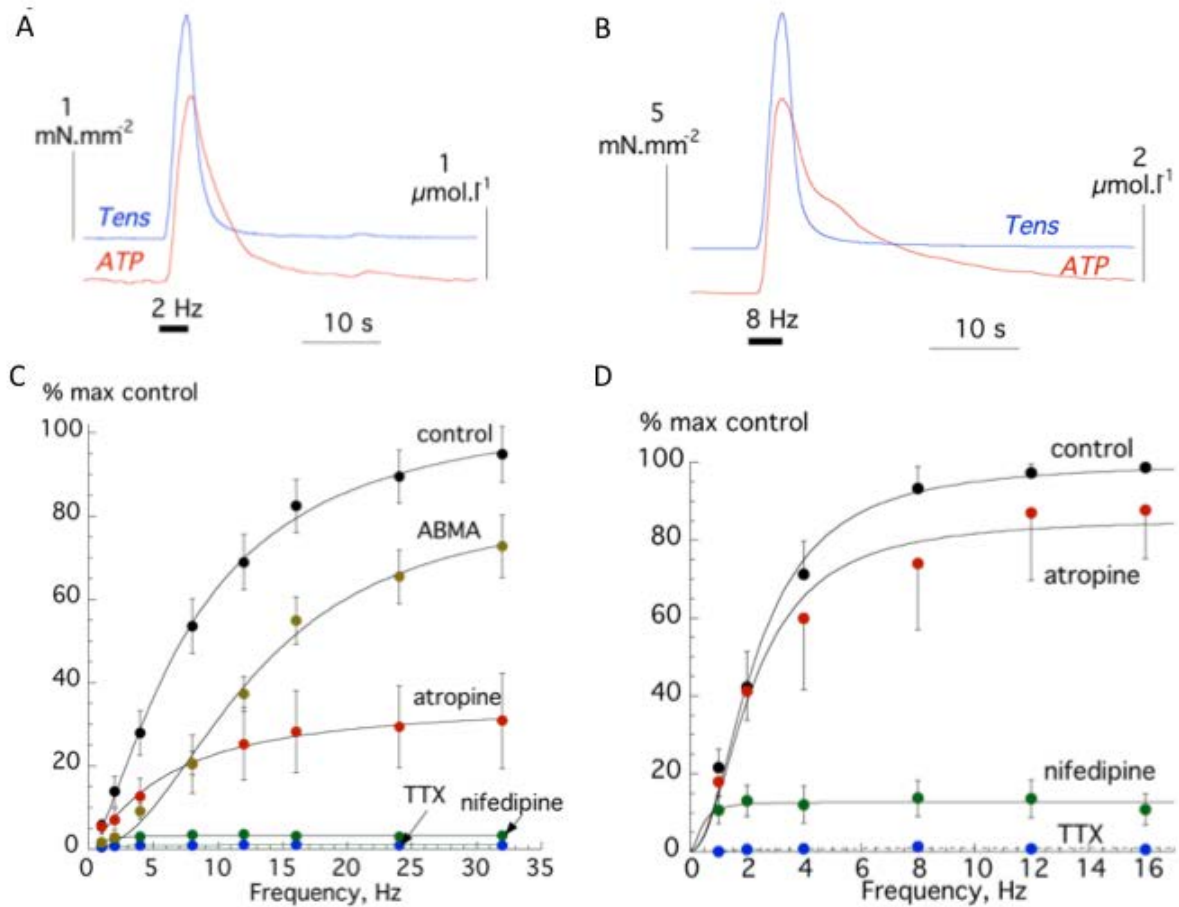
- Petkov, G.V. (2014). Central role of the BK channel in urinary bladder smooth muscle physiology and pathophysiology. *American Journal of Physiology, Regulatory, Integrative and Comparative Physiology* **307**: R571–R584. doi: 10.1152/ajpregu.00142.2014.
- Piriz, J., Rosato Siri, M.D., Pagani, R. & Uchitel, O.D. (2003). Nifedipine-mediated mobilization of intracellular calcium stores increases spontaneous neurotransmitter release at neonatal rat motor nerve terminals. *Journal of Pharmacology and Experimental Therapeutics* **306**: 658-663. doi: 10.1124/jpet.103.051524
- Sibley, G.N. (1984). A comparison of spontaneous and nerve-mediated activity in bladder muscle from man, pig and rabbit. *Journal of Physiology* **354**: 431–443. doi: 10.1113/jphysiol.1984.sp015386
- Sui, G.P., Coppen, S.R., Dupont, E., Rothery, S., Gillespie, J., Newgreen, D..... Fry, C.H. (2003). Impedance measurements and connexin expression in human detrusor muscle from stable and unstable bladders. *BJU International* **92**: 297-305.
- Wu, C., Sui, G. & Fry, C.H. (2002). The role of the L-type Ca<sup>2+</sup> channel in refilling functional intracellular Ca<sup>2+</sup> stores in guinea-pig detrusor smooth muscle. *Journal of Physiology* **538**: 357-369. doi: 10.1113/jphysiol.2001.013191
- Young, J.S., Meng, E., Cunnane, T.C. & Brain, K.L. (2008). Spontaneous purinergic neurotransmission in the mouse urinary bladder. *Journal of Physiology* **586**: 5743-5755. doi: 10.1113/jphysiol.2008.162040
- Zagorodnyuk, V.P., Gregory, S., Costa, M., Brookes, S.J., Tramontana, M., Giuliani, S. & Maggi, C.A. (2009). Spontaneous release of acetylcholine from autonomic nerves in the bladder. *British Journal of Pharmacology* **157**: 607-619. doi: 10.1111/j.1476-5381.2009.00166.x.

**Competing Interests.** The authors have no disclosures to report that pertain to this study

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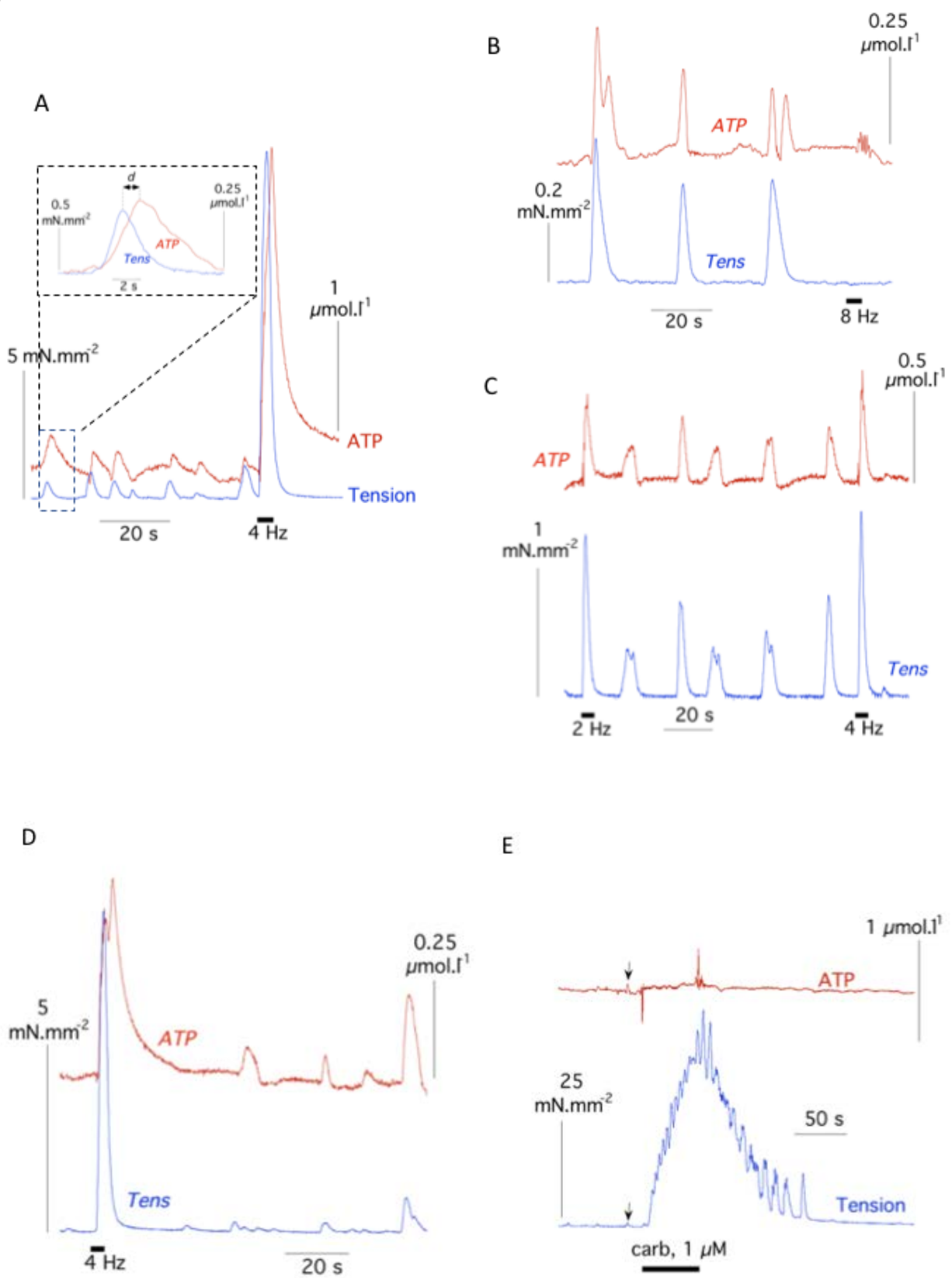
## Figure Legends

Figure 1



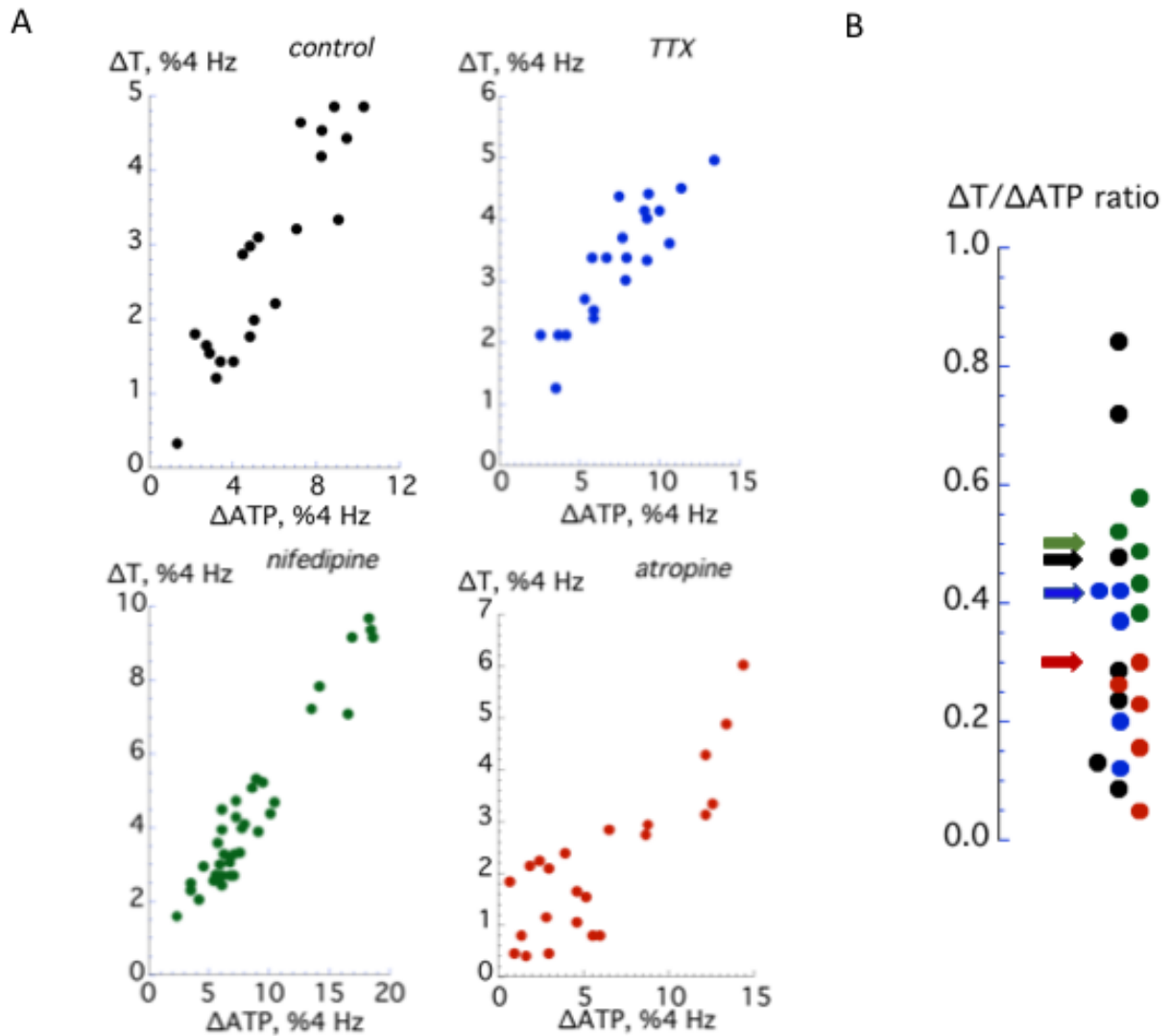
**Figure 1. Nerve-mediated tension and ATP transients.** A: Tension and ATP transients in response to electrical field stimulation at 2 Hz. B: Tension and ATP transients in response to electrical field stimulation at 8 Hz. C: Force-frequency relationships for guinea-pig detrusor under control conditions ( $n=12$ ) and in the presence of ABMA ( $n=5$ ), atropine ( $n=6$ ), nifedipine ( $n=5$ ), TTX ( $n=6$ ) or ABMA+atropine (atr,  $n=5$ ). All interventions at  $1 \mu\text{M}$ . Data (means $\pm$ SD) are fitted to equation 1). D: ATP amplitude-frequency relationships under control conditions ( $n=9$ ) and in the presence of atropine ( $n=6$ ), nifedipine ( $n=5$ ) or TTX ( $n=6$ ). Data (means $\pm$ SD) are fitted to equation 1).

Figure 2



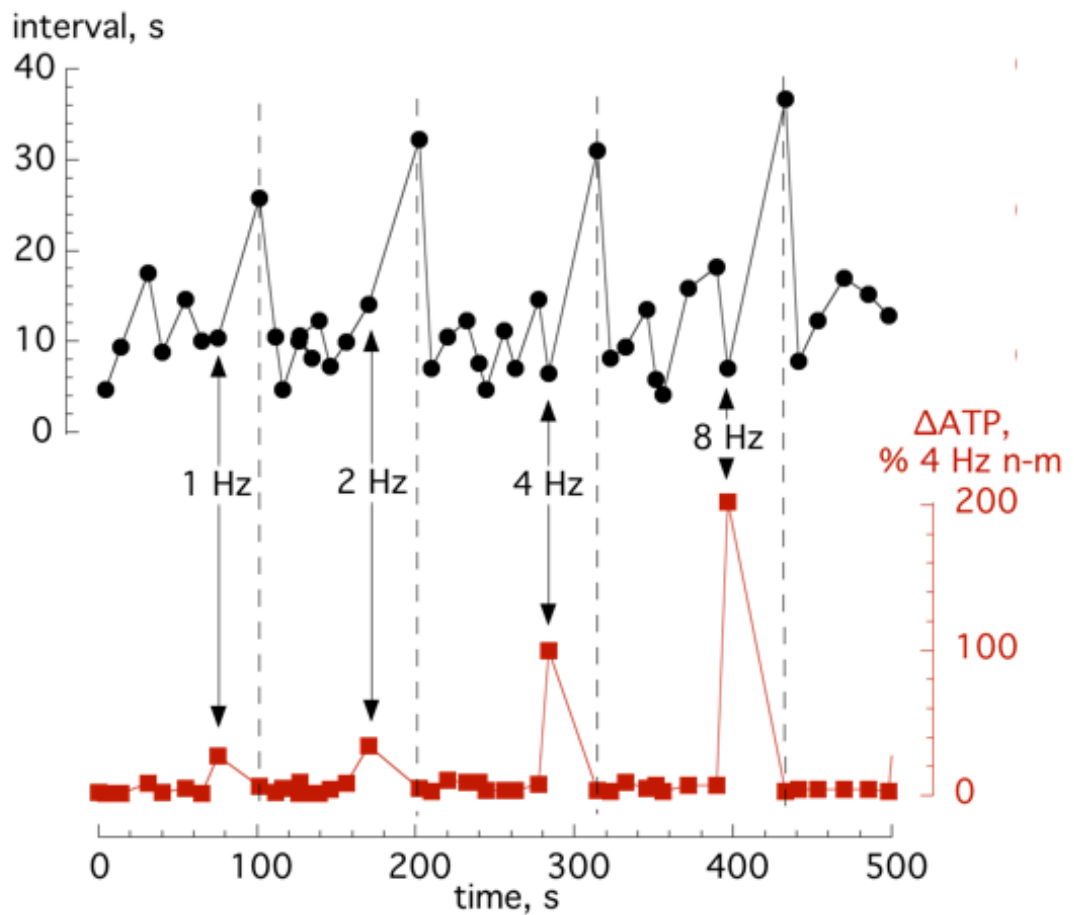
**Figure 2. Spontaneous tension and ATP transients.** A: Spontaneous transients in control conditions and (right) during 4 Hz stimulation. Spontaneous transients in any preparation are described as the percentage magnitude of that at 4 Hz stimulation. The first pair of transients is also shown in the inset to emphasise the delay, *d*, in the peak of the ATP transient. B: Spontaneous transients in the presence of 1  $\mu$ M TTX. Note the complex shape of some of the ATP transients. The right-hand side of the traces show where 8 Hz stimulation was applied without any evoked transients. C: Spontaneous transients in the presence of nifedipine, bounded by transients evoked at 2 and 4 Hz stimulation. D: Spontaneous transients in the presence of atropine. E: ATP electrode and contractile responses in the presence of 1  $\mu$ M carbachol

Figure 3

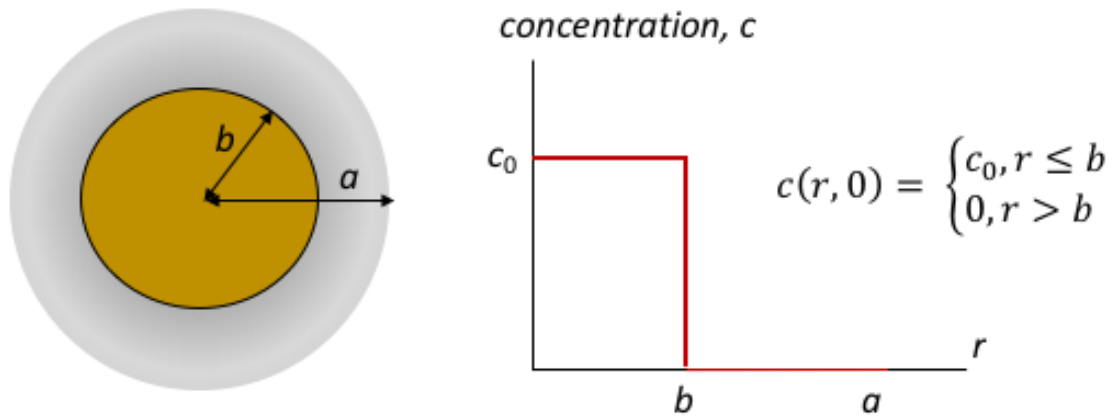


**Figure 3. Relationships between ATP and tension transients.** A: Plots of  $\Delta T$  as a function of  $\Delta ATP$  for transients from four preparations; in control conditions or in the presence of TTX, nifedipine or atropine, all interventions at 1  $\mu M$  concentration. Transient magnitudes are expressed as a percentage of those evoked at 4 Hz stimulation under control conditions. B: Dot plot of the slope values between  $\Delta ATP$  vs  $\Delta T$ ; data are under control conditions (black), with TTX (blue), nifedipine (green) or atropine (brown), the arrows correspond to values for the plots in part A.

Figure 4

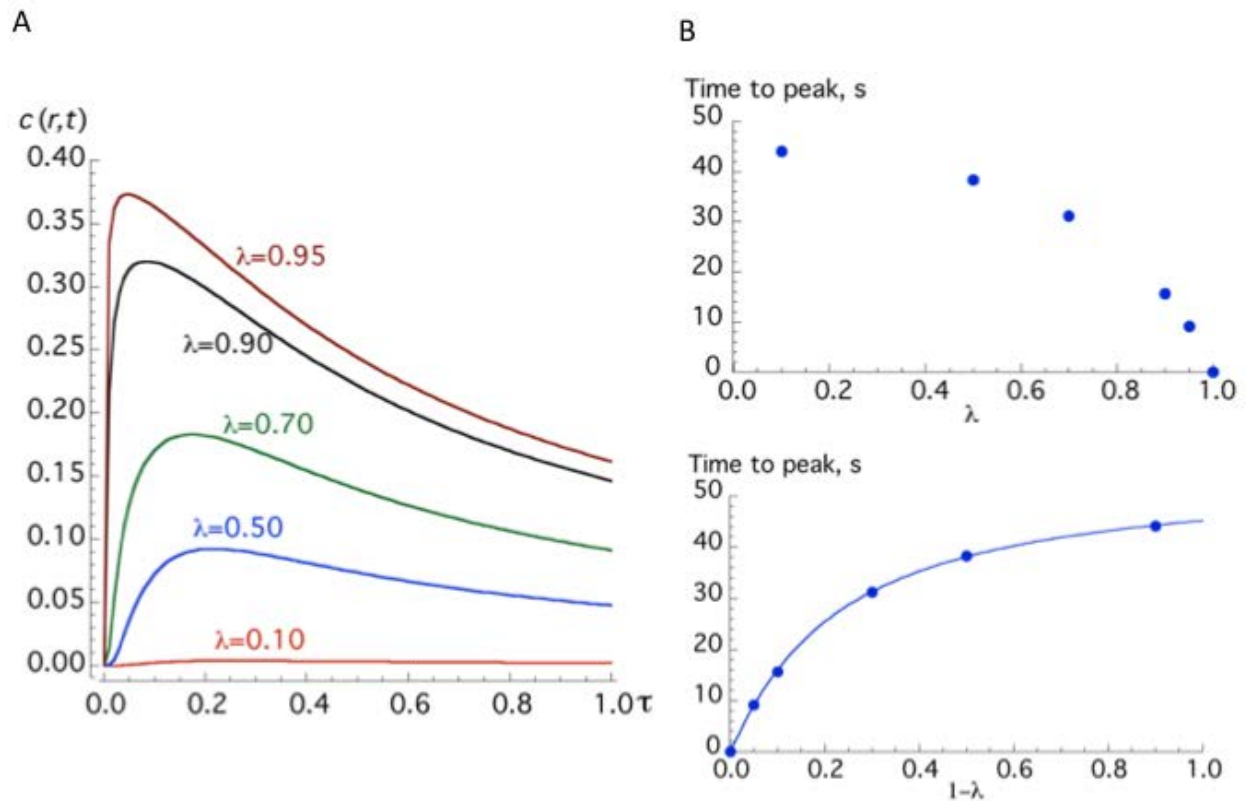


**Figure 4. Frequency of spontaneous ATP transients and nerve-mediated responses.** The top sequence (black) shows the interval between successive spontaneous ATP transients in the interval after larger nerve-mediated transient evoked by electrical field stimulation (EFS). The bottom sequence (brown) shows the magnitude of stimulated and spontaneous ATP transients. The vertical double-headed arrowed lines show when EFS occurred and the stimulation frequency. The dotted lines show the intervals between the EFS transient and the first spontaneous transient.



**Figure 5. The model of ATP diffusion from a detrusor preparation to a sensing electrode.** A muscle preparation of radius,  $b$ , surrounded by a fluid in which an ATP electrode is placed at distance,  $a$ . The initial concentration profile,  $c(r,t)$  at  $t=0$  is shown on the right.





**Figure 6. Solutions of the diffusion equation for ATP flux from a detrusor preparation.** A: the concentration profile,  $c(r,t)$ , for ATP diffusing from the boundary separating a muscle preparation, radius,  $b$ , and an ATP electrode near to the preparation in a superfusing solution at a distance  $a$  from the preparation centre. The different values of  $\lambda$  are the ratio  $b/a$ , i.e. smaller values denote greater distances of the electrode from the preparation. The abscissa label is either as the normalised variable  $\tau = Dt/a^2$ , or real time (sec) for a preparation of 0.04 cm radius and  $D_{37} = 9.2 \cdot 10^{-6} \text{ cm}^2 \cdot \text{s}^{-1}$ . B: upper plot; the time,  $t$ , to reach maximum concentration for ATP diffusing from the concentration profile in figure 5 for various values of  $\lambda$ . Lower plot;  $t$  plotted as a function of  $1-\lambda$  which permitted an empirical fit to  $y = (A \cdot x^n) / (B^n + x^n)$  for estimation of  $\lambda$ ; here,  $A = 54.01$ ,  $B = 0.224$ ,  $C = 1.083$ .