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Neurogenic and non-neurogenic responses in the urinary bladder of hibernating hamster

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1 Purinergic and cholinergic components of parasympathetic neurotransmission and contractile responses to exogenous α,β-methylene ATP, acetylcholine, substance K, substance P, calcitonin gene-related peptide, vasoactive intestinal polypeptide and capsaicin have been investigated in the urinary bladder of hibernating hamsters (4 weeks), cold exposed (4 weeks) and age-matched controls.

2 Electrical field stimulation (EFS) evoked increased frequency-dependent contractions in the detrusor strips from hibernating hamsters compared with those obtained from cold-exposed and age-matched animals. Tetrodotoxin (10^{-6} M) completely blocked the frequency-dependent contractions in all groups.

3 The purinergic component of the parasympathetic neurotransmission was not affected in hibernating and cold-exposed animals while the cholinergic component was increased with respect to age-matched animals. The neurogenic response to EFS, still present after incubation with atropine (10^{-6} M) and suramin (10^{-4} M), was attenuated by indomethacin (10^{-6} M) and blocked by tetrodotoxin (10^{-6} M).

4 Exogenous administration of α,β-methylene ATP elicited a significantly reduced contraction in strips from hibernating and cold-exposed hamsters relative to age-matched animals. The contractile response to exogenous acetylcholine was greater in the detrusors from hibernating hamsters than in cold-exposed and age-matched animals. Substance K elicited reduced contractions in preparations from hibernating animals compared with cold-exposed and control animals. Calcitonin gene-related peptide, vasoactive intestinal polypeptide, substance P and capsaicin did not elicit any relaxant or contractile response either at resting tone or in carbachol (5×10^{-7} M)-precontracted tissues.

5 In summary, our findings indicate that 4 weeks of hibernation can significantly increase neurogenic responses in the hamster urinary bladder. This appears to be due to an increase in postjunctional responses to acetylcholine. In contrast, there was a decrease of the postjunctional responses to the parasympathetic cotransmitter ATP and also to the sensory-motor neurotransmitter substance K.

Keywords: Hibernation; urinary bladder; purinergic neurotransmission; cholinergic neurotransmission

Introduction

It is well recognized that in pathological conditions, the innervation of the bladder and urethra can change: diabetic neuropathy, multiple sclerosis or spinal cord lesions affect the bladder and urethral function (Friedland & Perkash, 1983). Furthermore, even physiological processes can modify the innervation of the lower urinary tract (Lincoln & Burnstock, 1993). During pregnancy, the adrenergic fibres, that supply the reproductive organs as well as the lower urinary tract, undergo degenerative changes which protect the blood flow to the foetus. The adrenergic innervation of the bladder almost completely recovers 10 days after parturition (Qayyum *et al*., 1989). It was also shown that the adrenergic innervation of the detrusor muscle decreases with age in man (Gilpin *et al*., 1986). Vesicoureteral reflux is common in newborns because the innervation of the vesicoureteral junction is not fully developed (Kiruluta *et al*., 1986). In rats, the excitatory vesicovesical micturition reflex develops in parallel with the development of the substance P-containing sensory innervation of the bladder (Maggi *et al*., 1988).

During hibernation, which occurs in some mammals, a complex series of physiological events allows animals to survive in the season when there is a scarcity of food. The decrease in body temperature of hibernators from 37°C to 3°–5°C appears to be responsible for reducing all vital regulatory functions to a minimum compatible with life. It has been postulated that an accumulation or an absence of some 'critical substance' acting on the central nervous system leads to hibernation, although this substance has not yet been identified (Lyman & O'Brien, 1988). During hibernation, some organs like brain, heart and lungs continue to function, nevertheless the heart rate and the blood pressure are substantially decreased (Zatzman, 1984), the absence of feeding causes atrophy of the intestinal mucosa (Carey, 1995) and the urine flow of hibernators diminishes and in some cases ceases. In some hibernators like bears, the bladder wall transports water and solutes, such as urea, back into the blood at a rate that is approximately equal to their entry into the bladder (Nelson *et al*., 1975; Nelson, 1980), suggesting that the bladder plays a role in water and nitrogen conservation during hibernation. An ultrastructural observation of the bladder wall of a hibernating dormouse showed that the wall is thinner than in the active animals and that there are structural changes in the urothelium. These data suggest that hibernation can transiently affect the normal function of the urothelium and the bladder wall (Zancanaro *et al*., 1993). However, little information is available about the possible changes that can occur during hibernation in the autonomic innervation of the lower urinary tract.

The aim of this work was to study whether a seasonal physiological event like hibernation can affect the functional innervation and the responses to drugs in the hamster urinary

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bladder. Electrical field stimulation (EFS) was performed on bladders from hibernating animals and the data were compared with those obtained from age-matched controls and cold-exposed animals. In the present investigation we examined the effect of ATP and acetylcholine, which are considered to be the main neurotransmitters involved in parasympathetic control of bladder contraction (Burnstock *et al*., 1978; Burnstock, 1990; Theobald, 1995). The responses of the bladder to sensory-motor neurotransmitters such as substance P (SP), substance K (SK), calcitonin gene-related peptide (CGRP) and vasoactive intestinal polypeptide (VIP), which are responsible for sensory-motor control in the reflex of micturition (Maggi *et al*., 1987b), and capsaicin have also been tested.

Methods

Male golden hamsters (*Mesocricetus auratus*) 12 weeks old, weighing 120–140 g, were chosen for the study since these are non-seasonal hibernators, hibernation being induced entirely by photoperiod and external ambient temperature.

Induction of hibernation

Hamsters were initially placed in a LEC refrigerated incubator (model PL3) at a temperature of 20°C with a light : dark photoperiod of 8 : 16 h. The animals were caged 5 to a cage with food and water *ad libitum*. The temperature was gradually reduced by 1°C per day together with a reduction of 30 min of light per day until a temperature of 5°C and a photoperiod of 2 : 22 h was reached. The hamsters were then transferred to a cold room at a temperature of 5°C and 2 h of light per day. The hamsters were also housed individually at this point and nesting material was supplied. Food and water were supplied *ad libitum*. The hamsters remained in the cold room for 8–10 weeks before they started to hibernate. Once a hamster had gone into hibernation it was allowed to hibernate for 4 weeks before being killed. During this time the hamster underwent 'bouts' of hibernation lasting about 3–4 days, with periods of alertness in between. An animal was only killed while hibernating. Those hamsters that failed to hibernate after the 8–10 week period, or subsequently, were used as cold-exposed animals. In addition, age-matched controls were also used. Hibernation was judged by the lack of response to a physical stimulus, the sprinkling of sawdust onto the back of the hamster. A sleeping hamster always awoke to this stimulus, whereas a hibernating animal would not.

Tissue preparation and recording of mechanical activity

Hamsters were killed by asphyxiation with CO_2. Hibernating hamsters did not arouse before being killed. After death, rectal and cheek pouch temperatures were recorded, in addition to the weights of the animals. Bladders were quickly removed and opened by a ventral incision from the urethra to the dome. The bladder and the urethra were then separated by a transverse cut at the level of the bladder neck and 4 longitudinal detrusor strips (8 mm in length, 2 mm width and 13 ± 0.4 mg weight) were cut from each bladder. Each strip was threaded through a pair of platinum-ring electrodes (3 mm in diameter, 1 cm apart) connected to a Grass SD 9 stimulator, one end was attached to a holder and the other to a Dynamometer UF1 isometric force transducer coupled to a Grass 79 C polygraph. The strips were equilibrated for 1 h in 5 ml organ baths containing modified Krebs solution (mM: NaCl 133, KCl 4.7, $CaCl_2$ 2.5, NaH_2PO_4 1.4, $NaHCO_3$ 16.4, $MgSO_4$ 0.6, glucose 7.7) gassed with 95% O_2 and 5% CO_2 at 37 ± 0.5°C. The strips were initially loaded to a tension of 1 g (9.8 mN).

Experimental procedure

After the equilibration period, each preparation was exposed to acetylcholine (EC_{50}: 3×10^{-5} M) until 2 reproducible contractions were obtained. Frequency-response curves were then constructed: square wave pulses (70 V, 0.3 ms) were delivered for 30 s, at increasing frequencies (0.5–32 Hz), leaving a 4 min interval between two consecutive frequency steps. In order to evaluate purinergic and cholinergic components of parasympathetic neurotransmission, the tissues were then washed with fresh Krebs solution and exposed to atropine (10^{-6} M) or suramin (10^{-4} M). Since it is known that suramin is not a pure tool to work with (Crack *et al*., 1994), the purinergic component of parasympathetic neurotransmission was also evaluated after incubation of tissues with either pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS; 10^{-4} M) or after P2X receptor desensitization with 3 subsequent administrations of a high concentration (10^{-4} M) of α,β-methylene ATP (final concentration in the organ bath: 3×10^{-4} M): in both cases these drugs gave similar degrees of inhibition to that of suramin. In some preparations frequency-response curves were also performed in the presence of both antagonists or indomethacin (10^{-6} M) or tetrodotoxin (10^{-6} M). In parallel with the drug experiments, time controls were also performed by repeating the frequency response curves to EFS 3 times, without the administration of any antagonist. Non-cumulative concentration-response curves to acetylcholine (ACh; 10^{-7} M–10^{-3} M), α,β-methylene ATP (10^{-7} M–10^{-3} M) and SK (10^{-10} M–3×10^{-6} M) were performed in a different set of preparations. SP (10^{-9} M–10^{-7} M), CGRP (10^{-9} M–10^{-7} M), VIP (10^{-9} M–10^{-7} M) and capsaicin (10^{-6} M) were also examined in detrusor slips under resting tone and precontracted with carbachol (5×10^{-7} M, approximate EC_{50}).

Drugs used

Acetylcholine hydrochloride, atropine sulphate, calcitonin gene-related peptide, capsaicin, carbachol, indomethacin, α,β-methylene ATP lithium salt, substance K, substance P tetrodotoxin and vasoactive intestinal polypeptide were purchased from Sigma; suramin (Germanin) from Bayer. Pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) was a generous gift from Dr G. Lambrecht (University of Frankfurt, Germany).

Analysis of results

Contractile responses to exogenous drugs and the frequency-response curves to EFS were expressed as mN mg^{-1} wet tissue. Neurogenic responses in the presence of atropine and suramin were calculated as % of maximal responses (at 16 Hz) in the absence of the antagonist. Purinergic and cholinergic components were then calculated as difference between neurogenic responses in the absence and presence of suramin or atropine, respectively, and were expressed as percentage of response in the absence of antagonist at a given frequency. All data in the text are expressed as mean $\pm$ s.e.mean of at least 6 experiments. All curves were prepared with the help of the computer programme Prism: for each curve the programme calculates the lower and upper plateau, the slope, the EC_{50} and the pD_2 value $\pm$ s.e.mean. Concentration-response curves were compared by two-way analysis of variance (ANOVA) followed by Tukey-Kramer *post hoc* test (Ludbrook, 1994), pD_2 values and maximal responses to EFS or drugs were compared with a one way ANOVA followed by Tukey-Kramer *post hoc* test, by use of the computer program Minitab. A probability level less than 5% was regarded as significant.

Results

The proportion of hamsters that hibernated was 55%. The residual 45% of animals that failed to hibernate were used as cold-exposed. No differences were found in the bladder weight among the three groups: 125 ± 9, 116 ± 8 and 115 ± 10 mg in hibernating, cold-exposed hamsters and age-matched controls, respectively. The body weights were: 106 ± 2, 130 ± 9 and 136 ± 7 g (ANOVA, $P<0.05$, $n=16$); the rectal temperatures were: 12.6 ± 3, 32.7 ± 2 and 34.7 ± 1°C (ANOVA, $P<0.05$, $n=16$); the cheek pouch temperatures were: 14.4 ± 4, 33.1 ± 1 and 33.7 ± 1°C (ANOVA, $P<0.05$, $n=16$) in hibernating, cold-exposed and age-matched controls, respectively. The body weight, rectal and cheek pouch temperatures were significantly decreased in hibernating hamsters compared with cold-exposed and age-matched controls.

Contractile responses to EFS

EFS (0.5–32 Hz) produced greater contractions in detrusor strips of hibernating and cold-exposed hamsters compared with those obtained from age-matched controls. Tetrodotoxin (10^{-6} M) completely blocked the EFS-induced response at all frequencies (Figure 1). The frequency-response curve of hibernating hamster bladder differed significantly from those of cold-exposed (ANOVA, $P=0.048$, $n=7$) and age-matched control (ANOVA, $P=0.001$, $n=7$). The curve of cold-exposed significantly differed from the curve in age-matched control (ANOVA, $P=0.01$, $n=7$). Maximal responses were observed at 16 Hz (Table 1). The purinergic component of the parasympathetic neurotransmission evaluated as percentage of inhibition induced by suramin on EFS-induced response, is shown in Figure 2a. The percentage inhibition induced by suramin (10^{-4} M) was more evident at 0.5 Hz; 57.1 ± 7.0, 63.5 ± 8.0 and 40.9 ± 1.5, than at 32 Hz: 10.3 ± 4.4, 13.3 ± 3.0 and 11.2 ± 4.8 in hibernating, cold-exposed and age-matched control hamsters, respectively. The curves of the inhibition induced by suramin were not significantly different between the groups: hibernating vs cold-exposed (ANOVA, $P=0.63$, $n=7$) and vs age-matched control (ANOVA, $P=0.32$, $n=7$), cold-exposed vs age-matched control (ANOVA, $P=0.54$, $n=7$). The purinergic component of parasympathetic neurotransmission was also evaluated after the incubation of tissues with either PPADS (10^{-4} M) or after P2X receptor densenitization with 3 subsequent administrations of a high concentration (10^{-4} M) of α,β-methylene ATP; in both cases these drugs gave similar percentage inhibition to that induced by suramin. Moreover, suramin at the concentration used, did not alter the concentration-response curve to ACh.

Figure 2b shows the cholinergic component of the parasympathetic neurotransmission, evaluated as percentage of inhibition induced by atropine (10^{-6} M). The inhibition induced by atropine was significantly increased in hibernating (ANOVA, $P=0.001$, $n=7$) and cold-exposed (ANOVA, $P=0.01$, $n=7$) compared with age-matched controls. The inhibition induced by atropine in hibernating and in cold-exposed did not differ significantly (ANOVA, $P=0.14$, $n=7$).

The inhibition by suramin together with atropine on EFS-induced response is shown in Figure 2c. The percentage inhibition induced by both antagonists together was almost complete, especially at the lower frequencies of stimulation, being 88.9 ± 4.9, 97.2 ± 3.0 and 92 ± 7.0%, respectively, in hibernating, cold-exposed and age-matched control hamsters, with no significant difference between the groups (ANOVA, $P=0.10$, $n=6$). Figure 2d shows the percentage of inhibition of indomethacin alone and indomethacin together with suramin and atropine on EFS-induced response. The inhibition induced by indomethacin alone, was significantly greater in hibernating (ANOVA, $P=0.01$, $n=7$) than in cold-exposed and age-matched controls. The percentage of inhibition induced by indomethacin in cold-exposed and age-matched controls did not differ (ANOVA, $P=0.80$, $n=7$). Moreover, the inhibition induced by all antagonists together was complete at all frequencies of stimulation.

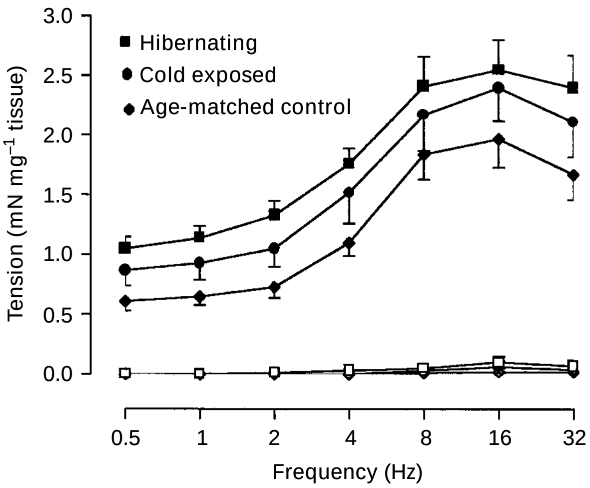

Figure 1 Frequency-response curves to EFS (70 V, 0.3 ms, 0.5–32 Hz) in longitudinal smooth muscle strips of urinary bladder in age-matched, cold-exposed and hibernating hamsters, before (solid symbols) and after tetrodotoxin (10^{-6} M) (open symbols). The curves were significantly different: hibernating vs cold-exposed (ANOVA, $P=0.048$, $n=7$); hibernating vs age-matched control (ANOVA, $P=0.001$, $n=7$); cold-exposed vs age-matched control (ANOVA, $P=0.01$, $n=7$). Tetrodotoxin completely blocked the response to EFS in all groups. Points show mean and vertical lines s.e.mean unless occluded by symbol.

Table 1 Maximal responses (mN mg^{-1} tissue) and pD_2 values to electrical field stimulation (EFS), α,β-methylene ATP (α,β-met ATP), acetylcholine (ACh) and substance K (SK) on urinary bladder of age-matched, cold-exposed and hibernating hamsters

	Age-matched control	*Cold-exposed*	*Hibernating*
EFS			
Max response	1.97 ± 0.24	2.40 ± 0.28	2.55 ± 0.25
α,β-met ATP			
pD_2	4.48 ± 0.14	4.64 ± 0.18	4.20 ± 0.17
Max response	1.27 ± 0.06	0.78 ± 0.05*	0.95 ± 0.07*
ACh			
pD_2	4.53 ± 0.19	4.73 ± 0.17	4.56 ± 0.22
Max response	1.63 ± 0.10	1.44 ± 0.07	2.24 ± 0.16†*
SK			
pD_2	7.95 ± 0.16	7.75 ± 0.16	8.26 ± 0.22
Max response	1.46 ± 0.09	1.61 ± 0.10	0.73 ± 0.05†*

Values shown are means ± s.e.mean of at least 6 experiments. *$P<0.05$ hibernating or cold-exposed vs control; †$P<0.05$ hibernating vs cold-exposed.

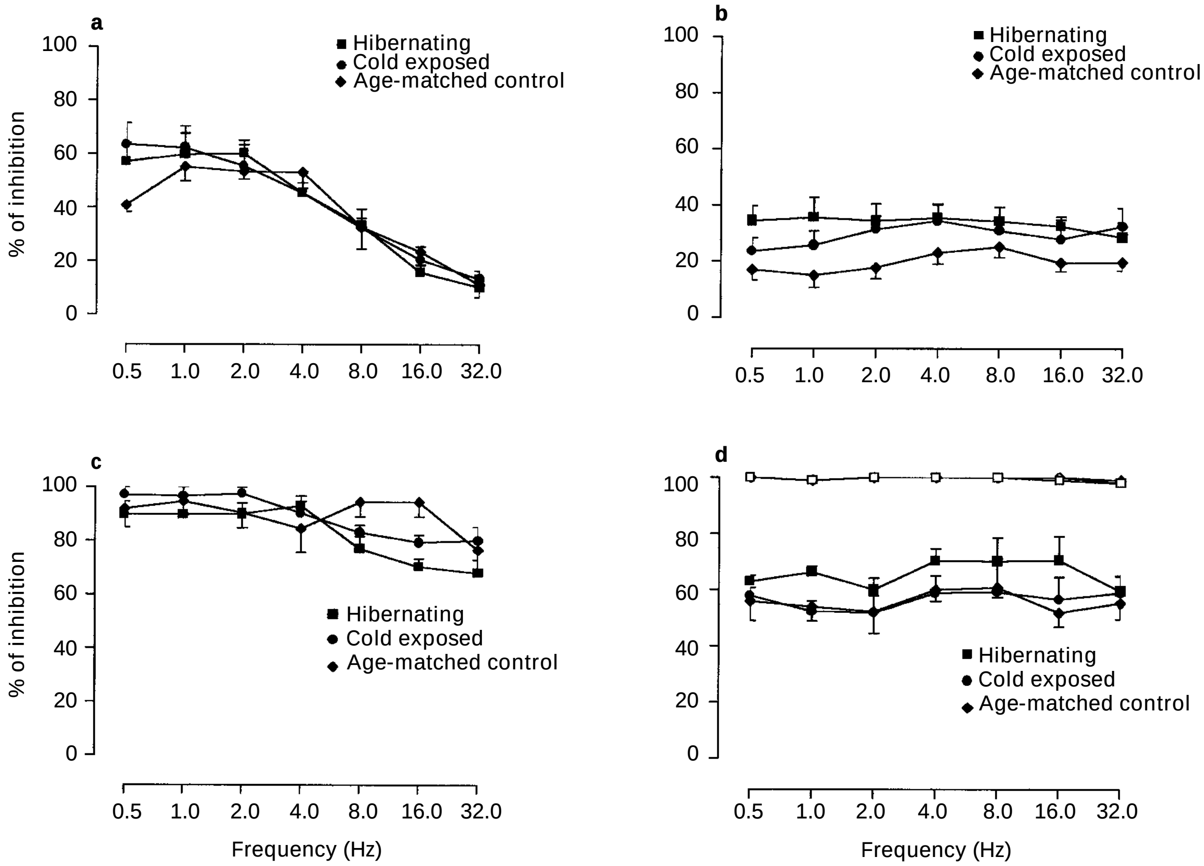

Figure 2 The inhibition (as %) provoked by (a) suramin, (b) atropine, (c) suramin plus atropine, and (d) indomethacin alone (solid symbols) and indomethacin plus suramin plus atropine (open symbols) on EFS-induced response in hibernating, cold-exposed and age-matched control hamsters. Suramin caused a similar inhibition among the groups. Atropine caused an increased inhibition in hibernating and cold-exposed animals: hibernating vs age-matched control (ANOVA, $P=0.001$, $n=7$); cold-exposed vs age-matched control (ANOVA, $P=0.01$, $n=7$). Indomethacin alone caused an increased inhibition in hibernating animals: hibernating vs age-matched (ANOVA, $P=0.01$, $n=7$), whereas treatment with indomethacin plus suramin plus atropine abolished neurogenic responses in all experimental groups. Points show mean and vertical lines s.e.mean unless occluded by symbol.

Contractile responses to exogenous α,β-methylene ATP and acetylcholine

α,β-Methylene ATP elicited concentration-dependent phasic contractions that were larger in strips from age-matched compared with cold exposed and hibernating hamsters (Figure 3a). The concentration-response curve of age-matched controls significantly differed from curves of cold-exposed (ANOVA, $P=0.001$; $n=6$) and hibernating animals (ANOVA, $P=0.002$; $n=6$). The pD_2 values were similar among the groups but maximal contractions to α,β-methylene ATP were significantly lower in cold-exposed and hibernating hamsters than in age-matched animals (Table 1).

Figure 3b shows the concentration-response curves to ACh; strips from hibernating hamsters exhibited greater contractions in response to ACh than strips from cold-exposed and age-matched hamsters. Concentration-response curves of age-matched and cold-exposed significantly differed from the curve of hibernating animals (ANOVA, $P=0.001$; $n=6$). The pD_2 values were similar among the groups and maximal contractions were increased in hibernating animals (Table 1). Contractions induced by KCl (120 mM) were greater in hibernating animals compared with the control groups: 2.2 ± 0.2; 2.1 ± 0.3 and 2.8 ± 0.4 mN mg^{-1} tissue, in age-matched, cold-exposed and hibernating hamsters, respectively, but the difference did not reach statistical significance.

Response to sensory-motor neurotransmitters

SK induced a rapid phasic and prolonged tonic activation of smooth muscle (Figure 4) and elicited concentration-dependent contractions that were larger in strips from age-matched and cold-exposed than in those from hibernating hamsters (Figure 4). Concentration-response curves of the age-matched and cold-exposed groups differed significantly from the curve of hibernating animals (ANOVA, $P=0.001$, $n=6$). The pD_2 values were similar among the groups and maximal contractions were reduced in hibernating hamsters (Table 1). Furthermore, SP (10^{-9} M–10^{-7} M), CGRP (10^{-9} M–10^{-7} M), VIP (10^{-9}–10^{-7} M) and capsaicin (10^{-6} M) did not elicit any contractile or relaxant response in detrusor strips, either at the resting tone or during carbachol (5×10^{-7} M) precontracted tone. Table 2 summarizes the results in cold-exposed and hibernating compared with age-matched hamsters.

Discussion

In hamster, as in other rodents such as guinea-pig (Kasakov & Burnstock, 1983), rabbit (Chancellor *et al*., 1992) and rat (Luheshi & Zar, 1990; Parija *et al*., 1991; Igawa *et al*., 1993), the neurogenic response of the detrusor muscle evoked by EFS consists of a cholinergic and a larger purinergic component.

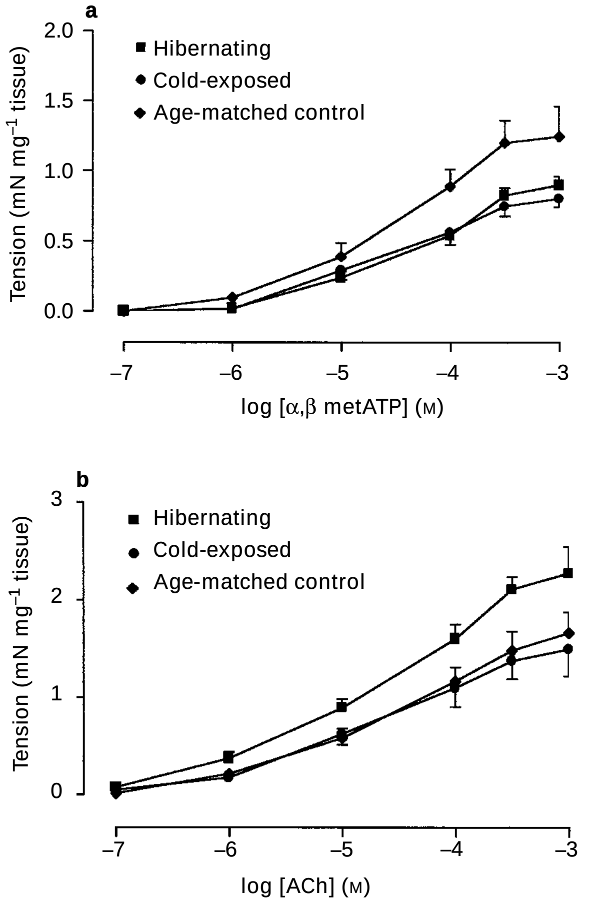

Figure 3 Non-cumulative concentration-response curves to (a) α,β-methylene ATP ($\alpha\beta$-metATP) and (b) acetylcholine (ACh) in longitudinal smooth muscle strips of urinary bladder in age-matched, cold-exposed and hibernating hamsters. α,β-MetATP elicited decreased contractions in cold-exposed and hibernating hamsters (ANOVA, $P<0.05$; $n=6$). ACh elicited increased contractions in hibernating hamsters (ANOVA, $P<0.05$; $n=6$) compared with those in age-matched and cold-exposed hamsters. Points show mean and vertical lines s.e.mean unless occluded by symbol.

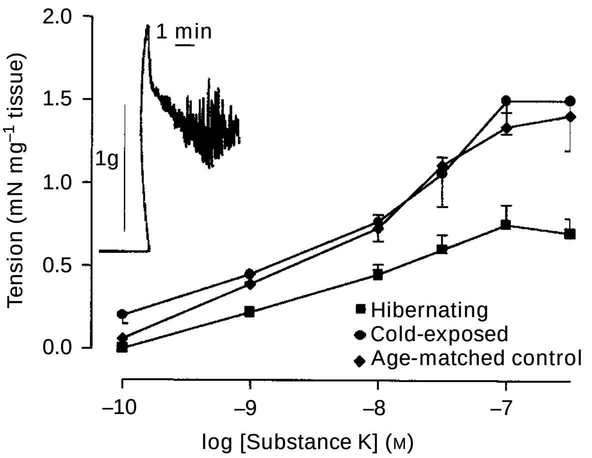

Figure 4 Typical tracing of the effect of substance K (10^{-6} M) on hamster bladder (inset). Non-cumulative concentration-response curves to substance K in longitudinal smooth muscle strips of urinary bladder in age-matched, cold-exposed and hibernating hamsters. Substance K elicited decreased contractions in hibernating compared with those in cold-exposed and age-matched hamsters (ANOVA, $P<0.05$; $n=6$). Points show mean and vertical lines s.e.mean unless occluded by symbol.

Table 2 Comparison of results obtained in bladder strips excised from cold-exposed and hibernating hamsters with those obtained in control animals

	Cold-exposed	*Hibernating*
Acetylcholine	=	↑
α,β-Methylene ATP	↓	↓
KCl	=	=
Substance K	=	↓
EFS	↑	↑
Inhibition by suramin	=	=
Inhibiton by atropine	↑	↑
Inhibition by indomethacin	=	↑

The up-arrow (↑) indicates an increased response, the down-arrow (↓) indicates a decreased response and the equal sign (=) indicates a response which was not significantly different from those obtained in control tissues.

Plasticity of the autonomic innervation supplying the lower urinary tract may occur in physiological conditions such as pregnancy (Alm *et al.*, 1979), development (Kiruluta *et al.*, 1986; Brauer *et al.*, 1994a, b) and ageing (Gilpin *et al.*, 1986).

The present study provides evidence for changes in the purinergic and cholinergic components of the parasympathetic neurotransmission in hamster urinary bladder, during a physiological event such as hibernation, which occurs in some animals. In our experimental conditions, the neurogenic response to EFS was significantly increased in bladder detrusor of hibernating and cold-exposed hamsters, compared with age-matched controls. The purinergic component of the parasympathetic neurotransmission, evaluated as percentage of inhibition induced by suramin, was similar in the three groups. Blockade or desensitization of P2X receptors by the purinoceptor anatagonist PPADS, or with α,β-methylene ATP also gave similar percentages of inhibition to that of suramin. The maximal response to exogenous α,β-methylene ATP was lower in hibernating and cold-exposed animals than in controls, but the pD_2 value of the concentration-response curve was not different from controls. The evaluation of contractile responses to α,β-methylene ATP indicated that a postjunctional decrease of purinoceptors may explain the data in hibernating and cold-exposed animals, whereas the similar pD_2 value indicates that the sensitivity of purine receptors probably did not change during the first month of hibernation or with cold exposure. A decreased number of purinoceptors in the hibernating and cold-exposed animals would be expected to elicit a smaller contraction in response to purinergic nerve stimulation if the same amount of ATP was being released. This did not occur. The response to purinergic nerve stimulation in the hibernating and cold-control animals was similar to the age-matched control group, which would seem to suggest that more ATP is being released from the parasympathetic nerves of the hibernating and cold-exposed bladders.

Since purinergic transmission in the urinary bladder is responsible for the initiation of micturition (Hoyle, 1994), an explanation for the postulated decrease in number of purinoceptors is that micturition may be reduced during hibernation. In fact, in hibernated animals such as the bear, the bladder plays a role in water and nitrogen conservation and transports water and solutes, such as urea, back into the blood (Nelson *et al.*, 1975; Nelson, 1980).

The cholinergic component of the neurogenic response, evaluated as percentage of inhibition induced by atropine, was significantly decreased in both cold-exposed and hibernating hamsters compared with age-matched controls. However, a significant increase of responsiveness to exogenous ACh was observed in hibernating but not in cold-exposed animals. Since the pD_2 values were similar for all the groups, these data suggest that postjunctional mechanisms, rather than prejunctional mechanisms may account for the larger cholinergic component in hibernating hamsters. For instance, an increased number of muscarinic receptors showing normal sensitivity could contribute to the larger responsiveness to ACh in hibernating hamsters. Alternatively, a decrease in ACh reuptake or an increased sensitivity of the effectors coupled to the muscarinic receptors, or an increase in ACh release in cold-exposed animals, may account for these data. Since the cholinergic activity is responsible for the maintenance and duration of micturition (Hoyle, 1994; Hoyle *et al*., 1994), one would expect a decreased cholinergic responsiveness rather than an increase of this activity during hibernation, thus confirming that changes in the autonomic innervation of the lower urinary tract, occcurring during hibernation, are not completely understood. Nevertheless, differentiation between cold-exposed and hibernating hamsters might also be related to differences in body temperature: 32–33°C in cold-exposed against 12–14°C in hibernating. It is possible that enzymes such as acetylcholinesterase and uptake mechanisms for ACh and/or ATP do not work properly at such low temperatures.

The percentage inhibition induced by atropine together with suramin was additive, and was about 90% of the neurogenic response. From these experiments it appears that there is no interaction between suramin and atropine in the hamster bladder. The neurogenic response was partially inhibited by incubation with indomethacin, whereas it was completely blocked in the presence of indomethacin, atropine and suramin. This observation suggests that in the hamster as in other mammals, ATP and ACh are cotransmitters in the urinary bladder and that prostaglandins are synthesized from the smooth muscle, as a result of parasympathetic nervous activity (Hoyle, 1994). The present study also suggests that prostanoids play a greater role in neurotransmission during hibernation. The fact that indomethacin alone reduced the neurogenic response by about 60% may be explained by considering that ATP-induced contractile responses in the bladder are partially due to prostaglandin synthesis (Hoyle, 1994). Since atropine plus suramin inhibited the neurogenic response by about 90%, it is more likely that prostaglandins are responsible for the remaining 10% of the contraction. There is also some evidence that prostanoids have a facilitatory role on purinergic neurotransmission. Bradykinin, which induces contraction partially by a release of prostanoids, is able to increase significantly the ATP-induced response in the rat bladder (Acevedo, 1990) and in guinea-pig vas deferens an analogue of prostaglandin I_2 was able to potentiate the ATP-induced responses (McKay & Poyser, 1995).

As the depolarizing effect of KCl did not significantly differ between the experimental groups, the observed changes cannot be ascribed to variations in the contractile machinery of the detrusor smooth muscle during hibernation and should be considered as specific and transient modifications in both purinergic and cholinergic responsiveness.

Exogenous sensory-motor peptides such as SP, SK, CGRP and VIP were also tested. Among these peptides, which are involved in the ‘efferent function’ of sensory-motor fibres in the rat bladder (Maggi, 1991), SK was the only one that elicited a contractile response in hamster bladder. SK is more active than SP in the activation of the micturition reflex in rats (Maggi *et al*., 1987b) and the SK concentration in the sensory fibres is inversely proportional to the volume threshold for micturition (Maggi *et al*., 1987a). The reduced responsiveness to SK exhibited by hibernating animals, could be explained by a decreased number of SK receptors showing normal sensitivity, which could be necessary to reduce micturition during the hibernation period. In our experiments capsaicin, CGRP, VIP and SP did not elicit any response both at the resting tone and in carbachol-precontracted hamster detrusor strips. This finding is in agreement with some studies involving tachykinin (Burcher & Buck, 1986) and vanilloid binding sites (Szallasi, 1994; Szallasi *et al*., 1993) in rodent bladder suggesting that in hamster, like in rabbit, the sensory fibres are different from those of the rat, mouse and guinea-pig.

In conclusion, our findings indicate that 4 weeks of hibernation can affect significantly neurogenic responses and those evoked by exogenous transmitters of the bladder in hamster. Some of these changes were also present in cold-exposed hamsters. EFS evoked larger contractions, more evident in hibernating that in cold-exposed hamsters, which suggests an increase in the postjunctional responses to ACh in hibernating hamsters. In contrast, a decrease in the postjunctional responses to exogenous α,β-methylene ATP is also postulated in both cold-exposed and hibernating hamsters. A decrease in the responsiveness to SK was also a feature of hibernating animals.

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