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Acute cerebral focal ischaemia alters the adrenergic and NANC responses in the bisected rat vas deferens

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1 Disturbances of the autonomic nervous system are common in right hemisphere stroke patients, including a marked decline in male sexual functions. There is a lack of information on the influence of stroke on male secondary sex organs such as the vas deferens.

2 This study investigates the effect of right brain focal ischaemia on the adrenergic and purinergic responses in isolated epididymal and prostatic portions of rat vas deferens.

3 In both epididymal and prostatic portions the concentration-response curves to noradrenaline are flattened resulting in a reduction (up to 67–76%) of the maximum contractile response in the tissue from ischaemic rats compared to the controls. In the prostatic portion from ischaemic rats the concentration-response curve to α,β-methylene ATP was also depressed.

4 The first purinergic and the second delayed adrenergic phase to single pulse was not modified by brain ischaemia. In contrast both phasic and tonic components of the electrically induced contractions by trains of stimuli at high frequencies (2–30 Hz) were significantly depressed in the epididymal and prostatic portions from ischaemic rats.

5 These results demonstrate an autonomic imbalance at the level of male sexual secondary organs which may contribute to sexual impairment after stroke.
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Keywords: Vas deferens; rat; purinergic transmission; noradrenergic transmission; stroke

Abbreviations: ATP, adenosine 5′ triphosphate; α,β-methylene ATP, alpha,beta-methylene adenosine 5′ triphosphate; MCAO, middle cerebral artery occlusion; NANC, non-adrenergic, non-cholinergic

Introduction

Disturbances of the autonomic nervous system are more common in right than in left hemisphere stroke patients (Korpelainen *et al*., 1999b; Coslett & Heilman, 1986; Agarwal & Jain, 1989). These impairments are attributed to damage of central autonomic control nuclei such as hypothalamus or to a lesion of cortical areas (Korpelainen *et al*., 1999b). The most relevant clinical problems include autonomic dysfunctions as abnormalities in the heart rate and blood pressure regulation, bladder dysfunction and a marked decline in male sexual functions, are most often complained about by stroke patients and their spouses (Coslett & Heilman, 1986; Korpelainen *et al*., 1994; 1998; 1999a). The impairment of sexual function might be due not only to psychological but also to organic causes and the present knowledge of the prevalence and clinical significance of these dysfunctions related to autonomic imbalance is still limited. Furthermore less attention has been paid to sexual dysfunctions linked to the alterations of male secondary sex organs such as vas deferens. Physiologically the semen ejection is due to rhythmic contraction of the longitudinal layers of smooth muscle of the vas deferens by producing an anterograde propulsion of the fluid. The contraction of vas deferens is directly due to adrenergic mechanisms but several endogenous factors, among which ATP play an important role.

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There is a lack of direct information on the influence of stroke on noradrenergic and NANC transmission in male secondary sex organs such as the vas deferens.

This study investigates the effect of brain ischaemia on the adrenergic and NANC responses in isolated epididymal and prostatic portions of rat vas deferens stimulated with exogenously applied or endogenously released neurotransmitters (noradrenaline and ATP). Finally, this experimental model can provide insight into the function of other smooth muscle that receive adrenergic and NANC innervation.

Methods

In vivo *rat treatments*

Photochemically-induced brain focal ischaemia Brain focal ischaemia was induced in Wistar–Harlan (250–350 g body weight) randomly selected rats anaesthetized by equitensine (3 ml kg^{-1}) in bolus to maintain rats in total anaesthesia during irradiation and to regain righting reflex within 1 h. Rose Bengal solution (1.7 mg 100 g^{-1}) was slowly loaded in the tail vein (3 ml kg^{-1}). An optical fibre was placed to produce ischaemia in the selected area of the right hemisphere, approximatively +2.0 mm from bregma and +3 mm laterally and irradiated by a halogen lamp (Endophare-Martin; 1.5 V, 150 W) for 30 min. Rectal temperature was

maintained at 37°C throughout the experiment by the use of a heated mat and lamp. Control rats received Rose Bengal injection but were not irradiated, since irradiation represented the ischaemic insult. In this experimental phase, the immobility of the animals was guaranteed by anaesthesia (Montalbetti *et al*., 1995). The rats were sacrificed 24 h after the ischaemic insult.

Middle cerebral artery occlusion

Middle cerebral artery occlusion (MCAO) was induced in rats by using a relatively non-invasive technique with an intraluminal filament, according to Zea Longa *et al*. (1989). Anaesthaesia was induced in Sprague–Dawley rats (270–300 g body weight) with 5% isoflurane in air and maintained with the lowest acceptable concentration of the anaesthetic (usually 2%). Body temperature was measured with a rectal probe and kept at 37°C with a heating pad. Under an operating microscope, the external and internal right carotid arteries were dissected and a silk suture was tied loosely around the external carotid stump. A silicone-coated nylon filament (diameter: 0.28 mm) was then inserted through the external into the internal carotid artery up to the Willis circle in order to occlude the middle cerebral artery. The silk suture was tightened around the intraluminal filament to prevent bleeding, then the animals were awakened from anaesthesia and returned to their cages MCAO rats were sacrificed 24 h after the ischaemic insult. The experimental protocol was carried out according to the Italian guidelines for animal care (DL 116/92) in application of the European Communities Council Directive (86/609/EEC) and was formally approved by the Animal Care Committee of the Department of Pharmacology of the University of Florence.

Isolated rat vas deferens preparations

Male Wistar–Harlan or Sprague–Dawley rats were sacrificed by CO_2 asphyxiation. Right and left vasa deferentia were quickly removed, cleaned from the connective tissue and placed in 20 ml organ bath filled with Krebs–Henseleit physiological solution of the following composition (mM): NaCl 118; KCl 5.6; $CaCl_2$ 2.5; $MgSO_4$ 1.19; $NaHPO_4$ 1.3; $NaHCO_3$ 25, EDTA 0. and (d)-glucose 10 mM, oxygenated (95% O_2–5% CO_2), heated at 37°C ± 0.5. Vasa deferentia were transversely bisected into two portions of equal length giving an epididymal and a prostatic half. Each tissue was placed in a 20-ml organ bath and tied at one end to the organ bath, the other end was connected to a Mangoni isometric transducer under a resting tension of 1 g. The tissues were equilibrated for at least 45 min with bath fluid changes every 10 min.

Experiments involving exogenous agonists

Cumulative concentration-response curves to noradrenaline (from 1×10^{-7} to 1×10^{-4} M) for epididymal and for prostatic portion were obtained by dosing at 0.5 log unit interval. Increasing concentrations were added to the organ bath after the response to the previous one had peaked. Concentration-response curves to α,β-methylene-ATP (from 1×10^{-7} to 1×10^{-4} M) were obtained in the epididymal and prostatic portion by dosing at 0.5-log unit intervals in a non-cumulative manner. Each concentration of α,β-methylene-ATP was added to the organ bath at intervals of 30 min. Preliminary experiments pointed out that no desensitization occurred within a 30 min washout period between two following administrations of each concentration of α,β-methylene-ATP and that the viability of the tissues was unaltered over a 5 h period. Each concentration of agonist was left in contact with the tissue until the response had peaked (3 to 30 s) and then the agonist was immediately washed out. A complete concentration response curve to noradrenaline or α,β-methylene-ATP was carried out with each vas deferens prepared from ischaemic or control rats. Preliminary experiments pointed out that Rose Bengal or anaesthetics used did not alter the cumulative concentration-response curve to noradrenaline and α,β-methylene-ATP compared to the untreated animals in different preparations evaluated by the comparison of the $-\log EC_{50}$ and by the E_{max} (data not shown).

Experiments involving transmural stimulation

Preliminary experiments pointed out that the smooth muscle cells were not being stimulated directly, since tetrodotoxin (1 μM, $n=4$) abolished the electrically induced tetanus at 15 Hz, confirming that the contraction of the preparation induced by field stimulation is neurogenic.

Single pulse field stimulation

Epididymal or prostatic portions of rat vas deferens were stimulated with a single stimulus (field stimulation, square waves, threshold voltage + 100%, 1 ms duration) to produce isometric contractions. Preliminary experiments indicated that no desensitization occurred with a rest interval of at least 10 min and that the response was reproducible over a period of 180 min in both control and ischaemic animals in both the tissues. After two control responses to single-pulse field stimulation were obtained, each tissue was equilibrated with a fixed concentration of test compound alone or in combination for at least 20 min before measurement of responses to single-pulse field stimulation. Only one concentration of each compound was tested on each tissue. The compounds tested were the α_1 adrenoceptor blocker prazosin and the P_{2x} purinoceptor antagonist suramin.

Frequency-response curves

Frequency-response curves (2–30 Hz) were obtained by stimulating the epididymal or prostatic portion of rat vas deferens with 30 s trains of pulses (1 ms duration) at supramaximal voltage of 10 Volt. A rest interval of 12 min was left between two following stimulations. Preliminary experiments pointed out that the reproducibility of the response at all the frequencies tested occurred in epididymal and prostatic portions from both control and ischaemic rats.

Drugs

The following drugs were used: (−)-noradrenaline bitartrate (Sigma), α,β-methylene-ATP lithium (Sigma). Noradrenaline (10^{-2} M) was dissolved in double-distilled water containing ascorbic acid (3 mg l^{-1}) and disodium edetate (1 mg l^{-1}) to prevent oxidation. α,β-methylene-ATP (10^{-2} M) was dissolved once a week, the stock solutions were kept frozen and

dilutions were made daily. Equitensine was prepared in sterile water for injection. Rose bengala (Sigma) was dissolved in sterile water for injection.

Statistics

Results are expressed as mean values ± s.e.mean. Statistical analysis of the results was performed using two-ways Anova for repeated data followed by Bonferroni's *t*-test (GraphPad Instat) or the Student's *t*-test for paired or unpaired data (Tallarida & Murray, 1987) were appropriate. $P<0.05$ was taken as the significance criterion. $-\text{Log } EC_{50}$ values for noradrenaline and α,β-methylene-ATP were calculated with GraphPadPrism (a data analysis package, San Diego, CA, U.S.A.) and expressed as means ± s.e.mean.

Results

The photochemical method induced a reproducible right frontoparietal focal ischaemic lesion. The maximum lesion area within the rostro-caudal extent of the injury was 4.5–5.5 mm^2 and the neuropathological evolution of this model of ischaemic stroke lesion reached the maximal extension within 24 h after irradiation. According to this protocol also MCAO rats were sacrificed 24 h after the ischaemic insult.

Responses to exogenous agonists

Preliminary experiments pointed out that Rose Bengal or anaestethics did not alter the normally quiescent tone of the preparations, furthermore the sensitivity to noradrenaline and α,β-methylene ATP, evaluated by the comparison of the $-\log EC_{50}$ and by the E_{max}, was not modified by the previous *in vivo* treatments (data not shown).

Photochemically-induced brain focal ischaemia

Addition of increasing amounts of noradrenaline (1×10^{-7}–3×10^{-4} M) and α,β-methylene-ATP (1×10^{-7}–1×10^{-4} M) produced a concentration-dependent increase in tension development using both the epididymal and prostatic portion of rat vas deferens. Figure 1 shows the cumulative concentration-response curve to noradrenaline in right and

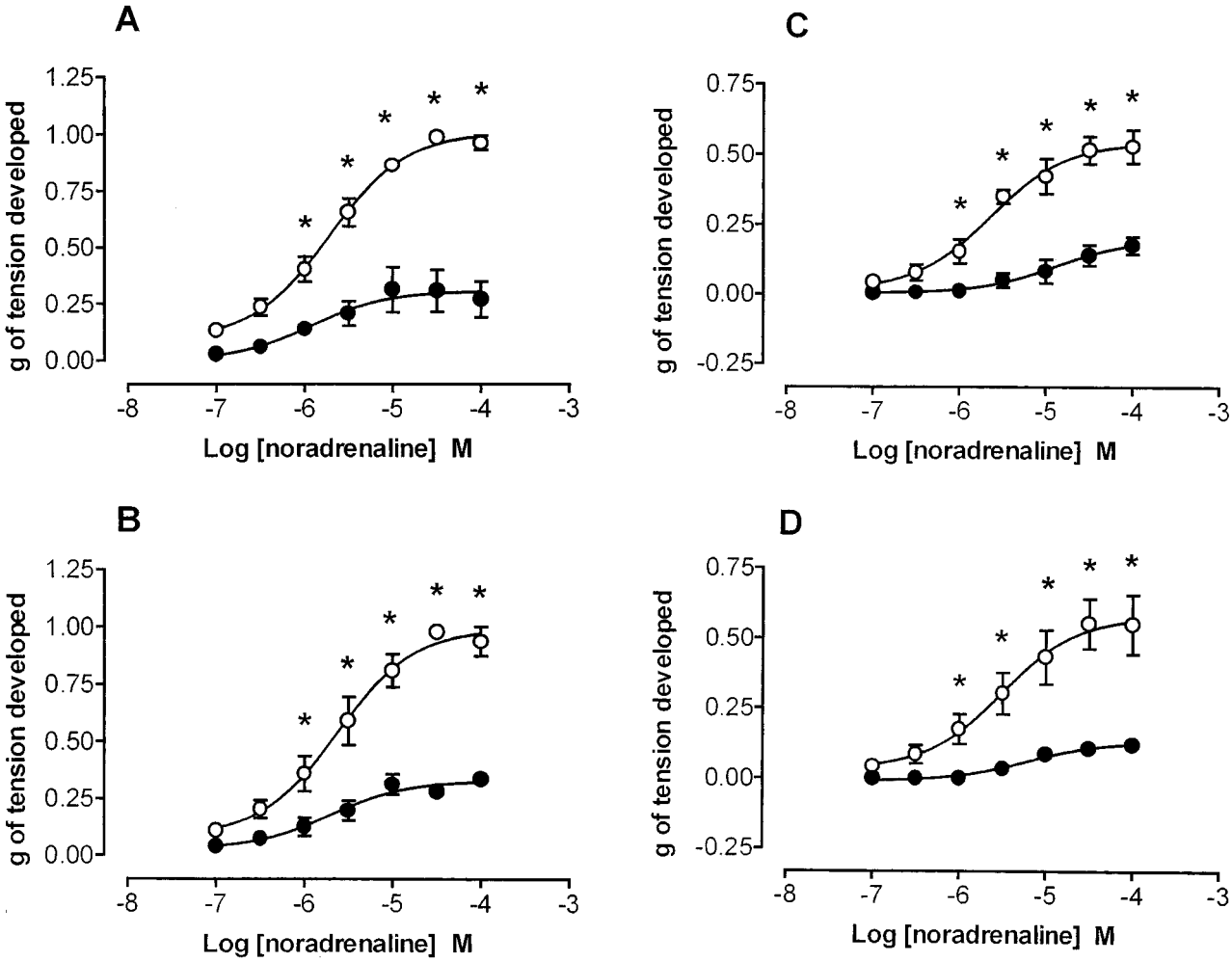

Figure 1 Mean concentration-response curves to noradrenaline obtained in right and left epididymal (A,B) and prostatic portions (C,D) from control (open circles) and ischaemic (focal photoinduced-ischaemia) rats (filled circles). Each point is the mean ± s.e.mean of 4–5 observations; where not visible the vertical bars lie within the sign. *Denotes a significant ($P<0.05$) difference between tissues from control and ischaemic rats.

left epididymal (Figure 1A, B) and prostatic portions (Figure 1C, D) from control and ischaemic rats.

A marked statistically significant decreased response to noradrenaline ($1\times10^{-6}-1\times10^{-4}$ M) was detected in both the epididymal and the prostatic portion in the tissues from ischaemic rats. The concentration-response curves were flattened resulting in a reduction up to 68.7% ± 5.49 (epididymal right, $n=4$, $P<0.05$) and 71.3% ± 2.95 (epididymal left, $n=5$, $P<0.05$) and in the prostatic portion up to 66.9% ± 4.85 (right, $n=4$, $P<0.05$) and 75.8 ± 6.06 left, $n=4$, $P<0.05$) of the maximum contractile response to noradrenaline in control tissues. The $-\log EC_{50}$ values calculated for the control tissues in the epididymal portions were: 5.7 ± 0.09 (right, $n=4$) and 5.6 ± 0.08 (left, $n=4$); 5.6 ± 0.16 (right, $n=4$) and 5.5 ± 0.11 (left, $n=4$) in the prostatic portions, within the usually observed values. No significant differences were found between right and left portions as regards agonist potency values in control tissues and on the inhibitory action exerted by the ischaemic insult.

The concentration-response curve to α,β-methylene-ATP in the epididymal portion of rat vas deferens from control and ischaemic rats were superimposable (Figure 2A). In the epidydidmal portion of rat vas deferens it was not possible to calculate the $-\log EC_{50}$ value for α,β-methylene-ATP because even with the highest concentration used (1×10^{-4} M) the curve had no clear maximum (Figure 2A) as previously observed with this tissue and stimulant (Grana *et al.*, 1997). At variance with the epididymal portion, a marked statistically significant decreased response to α,β-methylene ATP was detected in the prostatic portion from ischaemic rats compared to controls. The maximum contractile response to α,β-methylene ATP was depressed by 78.3% ± 4.70 (right, $n=4$, $P<0.05$) and 79.9 ± 4.90 (left, $n=4$, $P<0.05$). The $-\log EC_{50}$ value calculated in control tissues of 5.6 ± 0.10 (right, $n=4$) and 5.70 ± 0.10 (left, $n=4$) were not statistically different.

Experiments involving transmural stimulation

Single pulse field stimulation The mechanical response of the epididymal and prostatic portion of rat vas deferens to single pulse field stimulation was biphasic. It consisted of an early phase ($phase_I$) peaking at 250–300 ms followed by a second

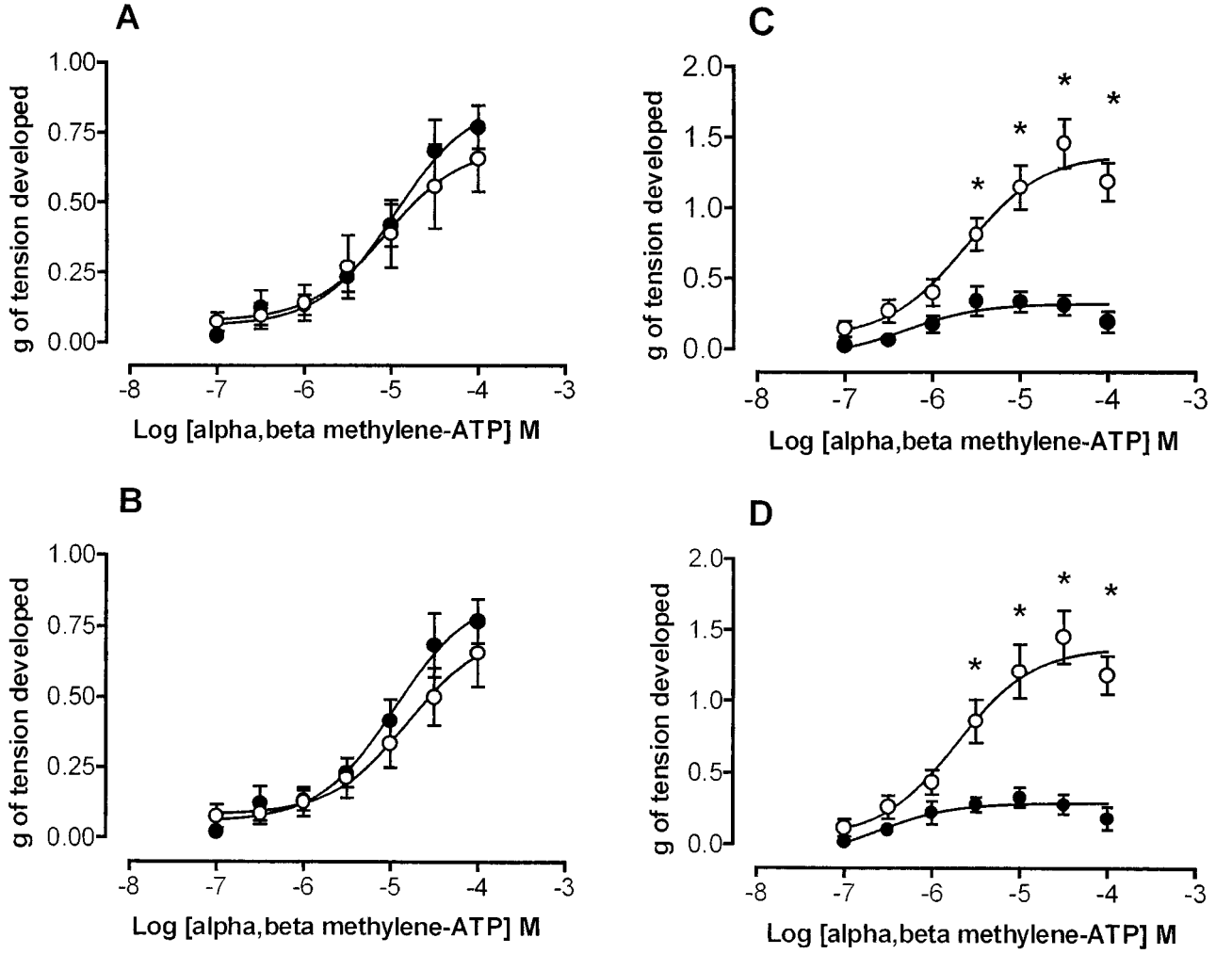

Figure 2 Mean concentration-response curves to α,β-methylene ATP obtained in right and left epididymal (A,B) and prostatic (C,D) portions from control (open circles) and ischaemic (focal photoinduced-ischaemia) rats (filled circles). Each point is the mean ± s.e.mean of four observations; where not visible the vertical bars lie within the sign. *Denotes a significant ($P<0.05$) difference between tissues from control and ischaemic rats.

slowly developing phase ($phase_{II}$) peaking at 650 ms, being the first purinergic phase prevalent in the prostatic portion and the second adrenergic one in the epididymal portion (Grana *et al*., 1997). Brain focal ischaemia did not modify the features of both phases to single pulse field stimulation as reported in Table 1. In both epididymal and prostatic portions of ischaemic and control rats the first phase was almost completely abolished by suramin 3×10^{-4} M (20 min, contact time) and the second one was completely suppressed by prazosin 1×10^{-8} M (20 min, contact time) as reported in Table 2. The combination of suramin and prazosin suppressed both phases (Table 2).

Frequency-response curves (2–30 Hz)

The response to trains of stimuli ranging from 2–30 Hz caused a frequency-dependent increase in tension developed in both epididymal and prostatic portions of rat vas deferens from control animals. The responses were typically biphasic consisting of a rapidly developing response (referred as phasic component) peaking at about 1 s and followed by a slow sustained one (referred as tonic component) peaking at about 7 s. Preliminary experiments pointed out that the frequency-response curve to 2 to 30 Hz was reproducible (data not shown).

Figures 3 and 4 show the mean frequency-response curves in right and left epididymal (Figure 3A,B,C,D) and prostatic (Figure 4A,B,C,D) portions from control and ischaemic rats.

In the epididymal portion of preparations from ischaemic rats, both phasic (Figure 3A,B) and tonic components (Figure 3C,D) were almost completely abolished ($P<0.05$) compared to the controls. Also in the prostatic portions both phasic (Figure 4A,B) and tonic (Figure 4C,D) contractions of electrically evoked tetanus were significantly depressed ($P<0.05$).

Middle cerebral artery occlusion induced ischaemia

In order to confirm the impairment following brain insult a second model of brain ischaemia, i.e. the middle cerebral artery occlusion (MCAO), was challenged on the responses produced by exogenously applied noradrenaline and α,β-methylene ATP. As reported in Figure 5A,B,C,D the response to exogenously applied noradrenaline was almost completely suppressed in both epididymal and prostatic portions of rat vas deferens, resulting in an inhibition of 73.0% ± 7.88 (epididymal right, $n=4$, $P<0.05$), 60.4% ± 7.20 (epididymal left, $n=4$, $P<0.05$), 66.5% ± 1.05 (prostatic right, $n=4$, $P<0.05$) and 74.5% ± 4.76 (prostatic left, $n=4$, $P<0.05$). $-\text{Log } EC_{50}$ in the right and left epididymal and prostatic portions in control tissues were 5.7 ± 0.07, 5.6 ± 0.09, 5.5 ± 0.16 and 5.5 ± 0.16, respectively. At variance with the photochemically induced ischaemia, a marked statistically ($P<0.05$) significant depression of the concentration-response curve to α,β-methylene ATP (1×10^{-5}–

Table 1 Effect of photochemically induced focal ischaemia on the contractile response to single-pulse field stimulation of the epididymal and prostatic portion of the rat vas deferens

	Epididymal portion		*Prostatic portion*	
Treatment	*Phase I*	*Phase II*	*Phase I*	*Phase II*
Control	0.14 ± 0.03 ($n=7$)	0.54 ± 0.06	1.05 ± 0.07 ($n=6$)	0.88 ± 0.11
Ischaemia 24 h	0.20 ± 0.06 ($n=9$)	0.54 ± 0.11	1.33 ± 0.11 ($n=6$)	1.13 ± 0.02

Results are expressed as absolute values (g).

Table 2 Antagonism by suramin and prazosin alone or in combination of the response to single field stimulation of the epididymal and prostatic portion of rat vas deferens from ischaemic and control rats

	Epididymal portion		*Prostatic portion*	
Treatment	*Phase I*	*Phase II*	*Phase I*	*Phase II*
Control				
Vehicle	98.27 ± 1.32 ($n=16$)	100.40 ± 1.58	105.80 ± 3.6 ($n=16$)	103.9 ± 5.2
Prazosin 1×10^{-8} M	96.27 ± 3.65 ($n=6$)	4.99 ± 4.99*	95.6 ± 4.8 ($n=6$)	0*
Suramin 3×10^{-4} M	0* ($n=6$)	98.56 ± 1.85	18.76 ± 5.8* ($n=6$)	106.1 ± 6.67
Prazosin 1×10^{-8} M + Suramin 3×10^{-4} M	0* ($n=4$)	8.39 ± 3.42*	12.8 ± 4.69* ($n=4$)	0*
Ischaemia 24 h				
Vehicle	92.05 ± 5.93 ($n=12$)	97.82 ± 8.88	99.01 ± 6.01 ($n=12$)	98.83 ± 3.99
Prazosin 1×10^{-8} M	98.63 ± 4.52 ($n=4$)	1.34 ± 0.78*	97.26 ± 3.35 ($n=4$)	0*
Suramin 3×10^{-4} M	3.12 ± 3.12* ($n=4$)	94.61 ± 5.32	21.22 ± 5.78* ($n=4$)	100.59 ± 3.43
Prazosin 1×10^{-8} M + Suramin 3×10^{-4} M	0* ($n=6$)	5.52 ± 2.12*	6.48 ± 4.26* ($n=6$)	8.62 ± 5.37*

Results are expressed as the percentage (means ± s.e.mean) of the response elicited in the absence of the test compound(s). *Student's *t*-test for paired data, $P<0.05$.

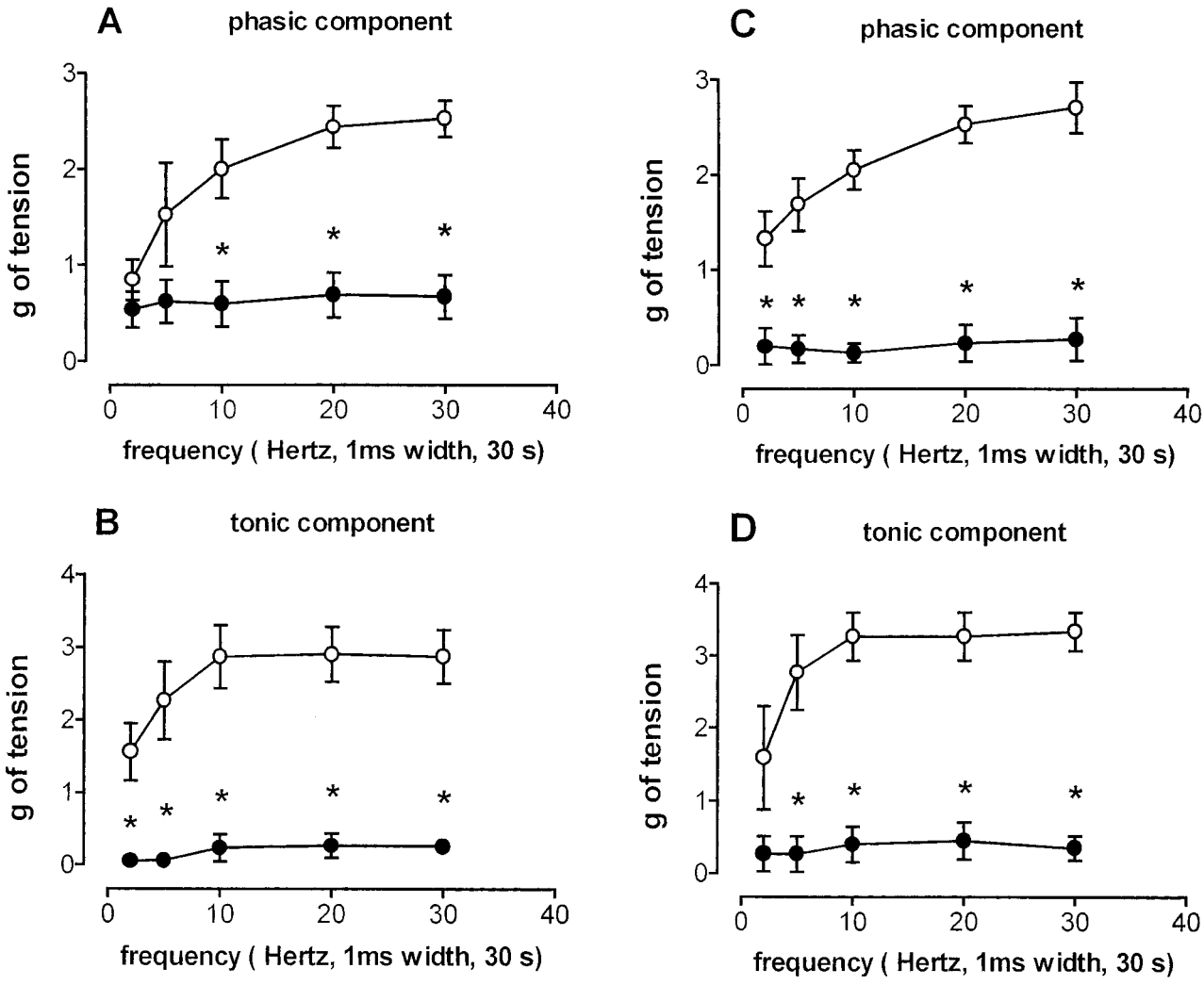

Figure 3 Frequency-response curves for electrical field stimulation in the right and left epididymal portions of rat vas deferens for phase I (phasic component, A,C) and phase II (tonic component, B,D) of the rat vas deferens from control (open circles) and ischaemic rats (filled circles). Each point represents the mean ± s.e.mean of 4–7 rats; where not visible the vertical bars lie within the sign. *Denotes a significant ($P<0.05$) difference between the responses from control and ischaemic rats.

1×10^{-4} M) was detected in right and left epididymal portions from MCAO rats, resulting in an inhibition of the highest concentration tested (1×10^{-4} M) of 82.3 ± 5.25 (right, $n=4$, $P<0.05$) and 70.5 ± 4.70 (left, $n=4$, $P<0.05$). Figure 6A,B,C,D shows the concentration-response curve to α,β-methylene ATP in the right and left prostatic portions from control and MCAO rats. The $-$Log EC_{50} from controls were calculated only in the right (5.8 ± 0.07, $n=4$) and left (5.8 ± 0.07, $n=4$) prostatic portions because no clear maximum could be detected in the epididymal portions as previously reported and illustrated in Figure 6A,B. The E_{max} values in prostatic portions of MCAO rats were significantly depressed up to 75.9% ± 4.09 (right, $n=4$, $P<0.05$) and 76.3% ± 3.90 (left, $n=4$, $P<0.05$).

Discussion and conclusions

The etiology of sexual dysfunction after stroke is multifactorial including both organic and psychosocial factors. Clinical studies described that stroke, especially when right hemisphere is involved, may cause sexual dysfunction and impotence although the ethiopathology remains unclear (Agarwal & Jain, 1989; Coslett & Heilman, 1986; Korpelainen *et al.*, 1998; 1999a, b). The data reported in the present paper show that brain focal ischaemia (both photochemical and MCAO induced-ischaemia) markedly impaired the response to noradrenaline and ATP in the epididymal and prostatic portion of rat vas deferens. In both tissues and both segments the response to exogenous noradrenaline was deeply depressed reaching a similar maximum (67–76%) inhibition in both the experimental models of focal ischaemia investigated. No differences were found in the response to exogenous noradrenaline between right and left portions of the same segment. As regards the response to exogenous ATP, a difference in the epididymal portion can be detected depending on the type of focal ischaemia considered. In rose Bengal ischaemic rats the response to α,β-methylene ATP was unaltered in both left and right epididymal portions, while in MCAO rats the concentration-response to exogenous ATP

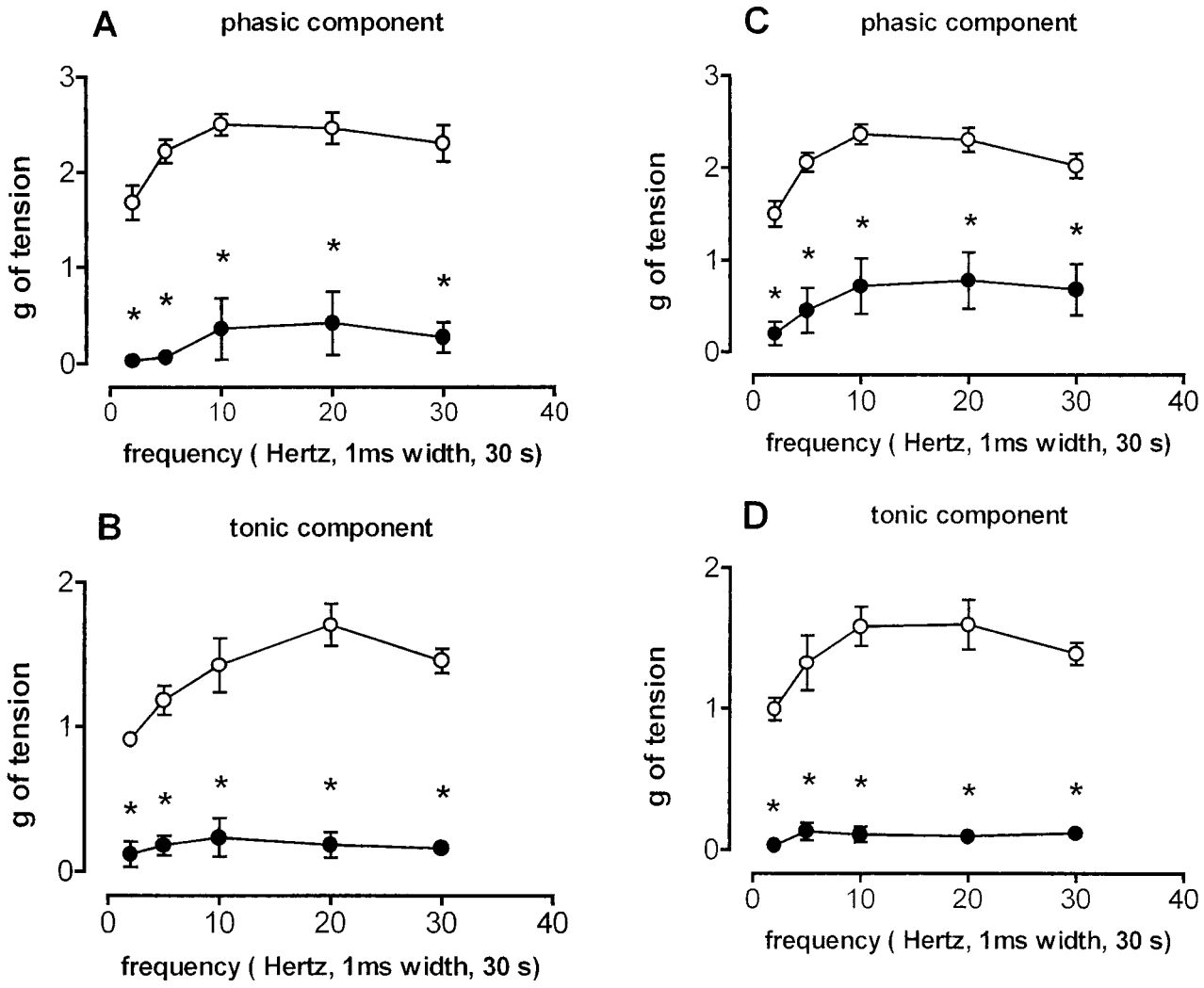

Figure 4 Frequency-response curves for electrical field stimulation in the right and left prostatic portions of rat vas deferens for phase I (phasic component, A,C) and phase II (tonic component, B,D) of the rat vas deferens from control (open circles) and ischaemic rats (filled circles). Each point represents the mean ± s.e.mean of 4–6 rats; where not visible the vertical bars lie within the sign. *Denotes a significant ($P<0.05$) difference between the responses from control and ischaemic rats.

was flattened in both right and left portions. These differences might be due to the methods used in the two experimental protocols and to a more extensive distribution of ischaemic damage produced by MCAO ischaemia. This seems to suggest that the severity in sympathetic dependent symptoms and in sexual impairment in right hemisphere stroke patients may depend upon the different etiology of brain vascular compromise and the extent of the lesion. A marked depression (70–83%) of the response in the prostatic portions in both the experimental models of focal brain ischaemia was detected and again the same pattern of behaviour was found the right and left portions. These results suggest an impairment of adrenergic and NANC component at postsynaptic level in the vas deferens.

Furthermore we evaluated the influence of acute photochemical ischaemia on the electrically evoked contractions produced by the endogenously released noradrenaline and ATP to single pulse and trains of stimuli (2–30 Hz) in both epididymal and prostatic portions. No significant differences were detected for phase I and phase II of single pulse field stimulation in the epididymal and prostatic portions from ischaemic and control rats. Furthermore the combination of prazosin (1×10^{-8} M) and suramin (3×10^{-4} M) completely abolished both phases in both tissues from ischaemic and control rats suggesting that at 24 h after stroke no impairment occurred at presynaptical level. The lack of impairment of the responses to single shock seems to suggest also that the $\alpha_{1D/L}$ adrenoceptors selectively activated postsynaptically by noradrenaline released by single pulse were not modified (Aboud *et al*., 1993; Ohmura *et al*., 1992; Docherty, 1998).

The response to trains of stimuli at high frequencies of both portions of rat vas deferens is biphasic too (Swedin, 1971). The features of the first and second phases of tetanus differ markedly from those of single pulse in that both phases consist of an adrenergic and an α-adrenoceptor independent component throughout its duration (Swedin, 1971; McGrath, 1978; Brown *et al*., 1983; Amobi & Smith, 1987). Acute photochemical ischaemia almost completely suppressed both phasic and tonic contractions in both right and left

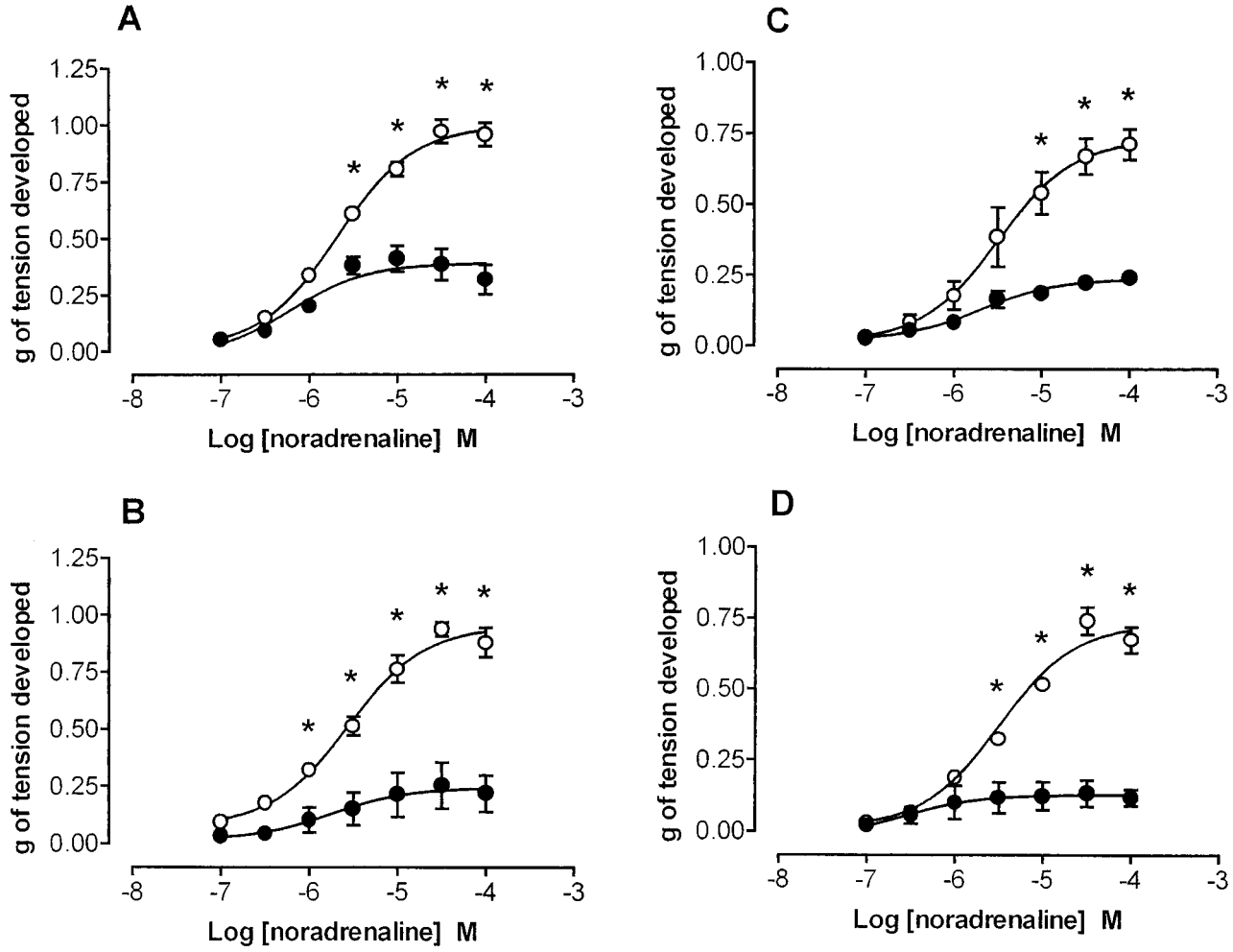

Figure 5 Mean concentration-response curves to noradrenaline obtained in right and left epididymal (A,B) and prostatic portions (C,D) from control (open circles) and MCAO (middle cerebral artery occluded) rats (filled circles). Each point is the mean ± s.e.mean of four observations; where not visible the vertical bars lie within the sign. *Denotes a significant ($P<0.05$) difference between tissues from control and ischaemic rats.

epididymal and prostatic portions of vas deferens. Again no different impairment was found between right and left portions. This inhibition was similar to that observed with the exogenously applied noradrenaline and ATP. The data reported in the present paper provide for the first time evidence that a stroke strongly affected the contractile activity of the vas deferens, possibly contributing to impaired sexual function.

The mechanisms underlying this effect of brain focal ischaemia on the epididymal and prostatic portions of the rat vas deferens appear very complex. The depressed responses to exogenous noradrenaline or trains of stimuli at high frequency seem to suggest that a postsynaptical impairment occurred at least when a large amount of neurotransmitter(s) reaches the junctional cleft by exogenous addition or released by electrically applied tetanus interacting with the α_{1A}-adrenoceptor and P_{2x}- purinoceptor subtypes (Han *et al*., 1987; Amobi *et al*., 1999). On the other hand the failure to contract of both the segments to trains of stimuli at high frequency may not exclude the involvement of an additional prejunctional mechanism, i.e. a decrease in neurotransmitter(s) release.

Another possible explanation of the lack of contraction on the vas deferens is that brain focal ischaemia can affect the neuroendocrine system in the rat as shown in men (Jeppesen *et al*., 1996). The response and the activity of the rat vas deferens depend indeed on an intact neuroendocrine system (Wakade *et al*., 1975; Preslock & McCann, 1985). Jeppesen *et al*. (1996) found that total and free serum testosterone concentrations were significantly decreased in men 1 day after stroke onset. Furthermore, Keast & Suders (1998); Keast (1999) reported that circulating testosterone is essential for the maintenance of the noradrenergic pelvic neurons that supply vas deferens although less direct actions such as effect on target organs or neurotrophic factors released by testosterone cannot be excluded. In a preliminary study Stoleru *et al*. (1999) found that specific cortical areas such as frontotemporal and anterior cingulate cortex are selectively involved in sexual response. Some of these areas are involved in the control of neuroendocrine and autonomic responses and are positively correlated to the serum testosterone levels in healthy men. The impairment of vas deferens contractile responses was observed 24 h after ischaemia, time at which no changes in organ weight or basal activity have been so far

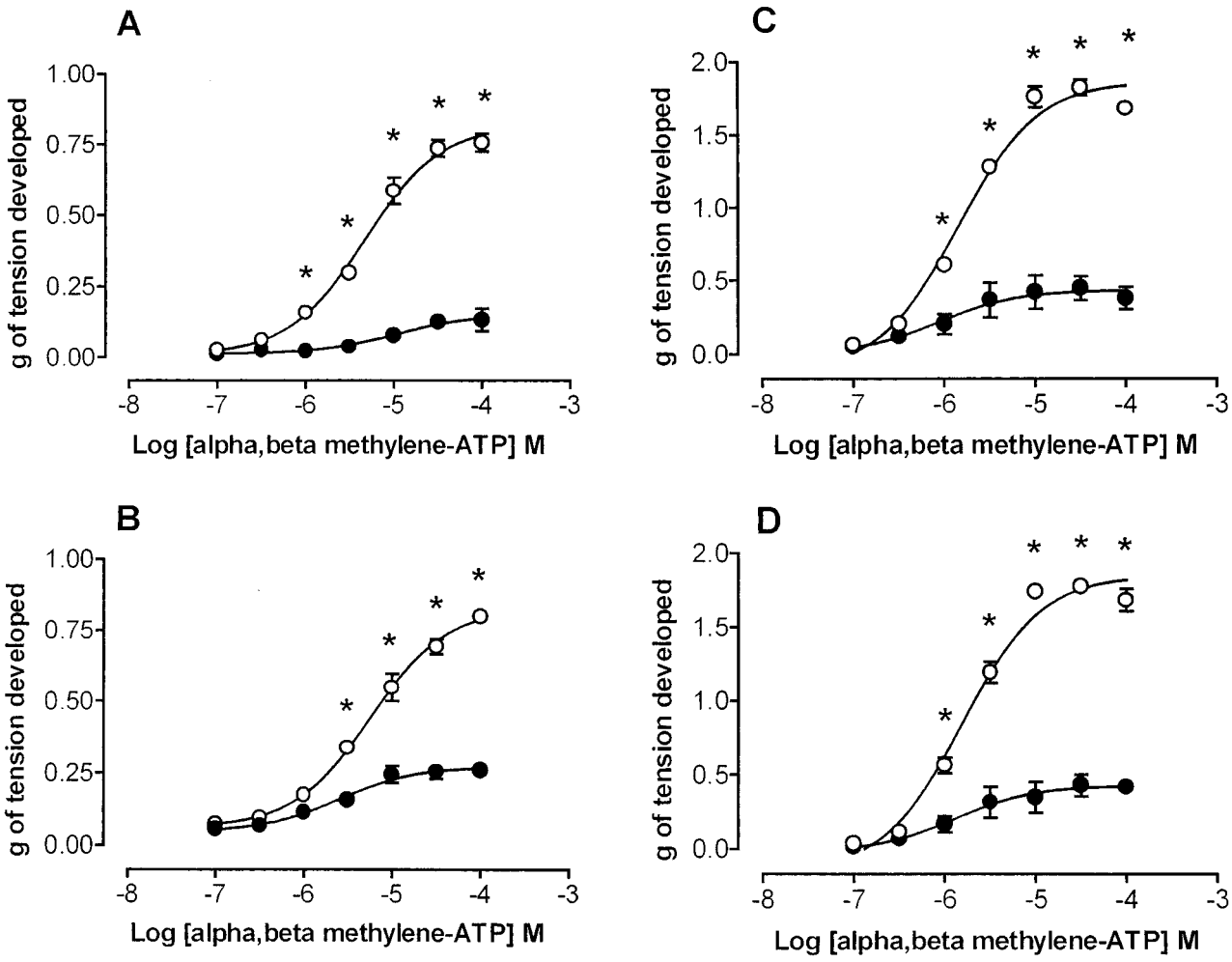

Figure 6 Mean concentration-response curves to α,β-methylene ATP obtained in right and left epididymal (A,B) and prostatic (C,D) portions from control (open circles) and MCAO rats (filled circles). Each point is the mean $\pm$ s.e.mean of four observations; where not visible the vertical bars lie within the sign. *Denotes a significant ($P<0.05$) difference between tissues from control and ischaemic rats.

detected. These results seem to suggest that cortical stroke dysrupts the descending pathways that physiologically modulate the physiological rhytmic contractions of vas deferens deeply affecting the ability of this tissue to contract, altering the seminal emission and ultimately fertility.

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