

## RESEARCH PAPER

# High concentrations of dexmedetomidine inhibit compound action potentials in frog sciatic nerves without $\alpha_2$ adrenoceptor activation

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## BACKGROUND AND PURPOSE

Dexmedetomidine, an  $\alpha_2$ -adrenoceptor agonist, exhibits anti-nociceptive actions at the spinal cord and enhances the effect of local anaesthetics in the peripheral nervous system. Although the latter action may be attributed in part to inhibition of nerve conduction produced by dexmedetomidine, this has not been fully examined yet.

## EXPERIMENTAL APPROACH

We examined the effects of various adrenoceptor agonists including dexmedetomidine, and tetracaine, a local anaesthetic, on compound action potentials (CAPs) recorded from the frog sciatic nerve, using the air-gap method.

## KEY RESULTS

Dexmedetomidine reversibly and concentration-dependently reduced the peak amplitude of CAPs ( $IC_{50} = 0.40 \text{ mmol}\cdot\text{L}^{-1}$ ). This action was not antagonized by two  $\alpha_2$ -adrenoceptor antagonists, yohimbine and atipamezole; the latter antagonist itself reduced CAP peak amplitude. Clonidine and oxymetazoline, two other  $\alpha_2$ -adrenoceptor agonists, also inhibited CAPs; the maximum effect of clonidine was only 20%, while oxymetazoline was less potent ( $IC_{50} = 1.5 \text{ mmol}\cdot\text{L}^{-1}$ ) than dexmedetomidine. On the other hand, ( $\pm$ )-adrenaline, ( $\pm$ )-noradrenaline,  $\alpha_1$ -adrenoceptor agonist (-)-phenylephrine and  $\beta$ -adrenoceptor agonist (-)-isoprenaline (each  $1 \text{ mmol}\cdot\text{L}^{-1}$ ) had no effect on CAPs. Tetracaine reversibly reduced CAP peak amplitude ( $IC_{50}$  of  $0.014 \text{ mmol}\cdot\text{L}^{-1}$ ).

## CONCLUSIONS AND IMPLICATIONS

Dexmedetomidine reduced CAP peak amplitude without  $\alpha_2$ -adrenoceptor activation (at concentrations >1000-fold higher than those used as  $\alpha_2$  adrenoceptor agonist), with a lower potency than tetracaine. CAPs were inhibited by other  $\alpha_2$  adrenoceptor agonists, oxymetazoline and clonidine, and also an  $\alpha_2$  adrenoceptor antagonist atipamezole. Thus, some drugs acting on  $\alpha_2$  adrenoceptors are able to block nerve conduction.

## Abbreviations

AP, action potential; CAP, compound action potential; CV, conduction velocity; DRG, dorsal root ganglia; HPD, half-peak duration; SG, substantia gelatinosa; TTX, tetrodotoxin

## Introduction

$\alpha_2$ -Adrenoceptor agonists have a variety of clinical actions including vasoconstriction, sedation, anal-

gesia, anaesthesia and anaesthetic-sparing effects (for review see Yaksh, 1985; Kamibayashi and Maze, 2000). For example, intrathecal or epidural administration of  $\alpha_2$  adrenoceptor agonists such

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## Keywords

dexmedetomidine; tetracaine; local anaesthetic; compound action potential; sciatic nerve; frog; adrenoceptors

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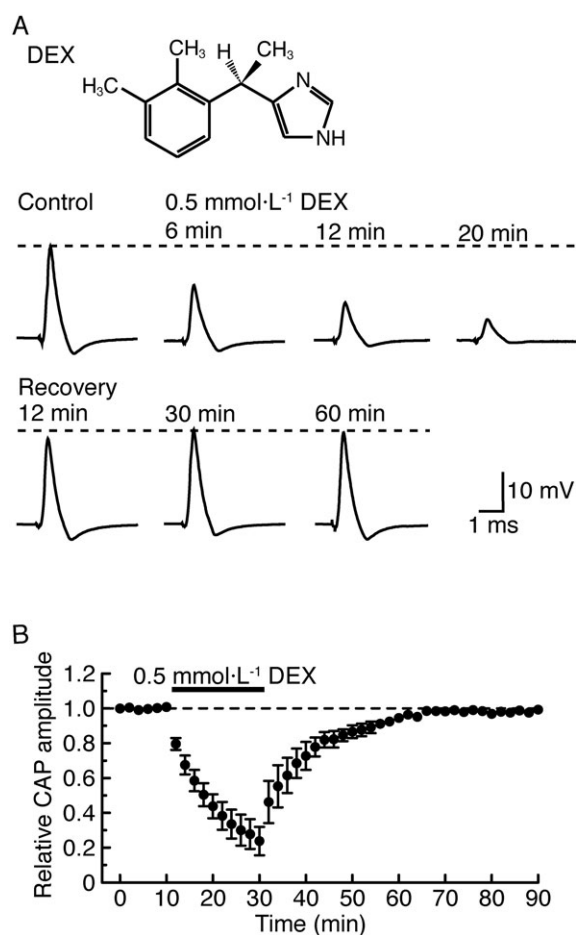
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**Figure 1**

$\alpha_2$ -Adrenoceptor agonist dexmedetomidine (DEX;  $0.5 \text{ mmol}\cdot\text{L}^{-1}$ ) reversibly reduces the peak amplitude of compound action potentials (CAPs) recorded from frog sciatic nerve fibres. (A) Recordings of CAPs in the control, after 6, 12 and 20 min of exposure to dexmedetomidine and thereafter 12, 30 and 60 min in the absence of dexmedetomidine. Upper inset shows the chemical structure of dexmedetomidine (see Bhana *et al.*, 2000). (B) Average time course of changes in CAP peak amplitudes following exposure to dexmedetomidine for 20 min, relative to that before the soaking, obtained from five sciatic nerves. The dexmedetomidine effect was close to a steady effect after 20 min of exposure.

as clonidine and dexmedetomidine ((+)-(S)-4-[1-(2,3-dimethylphenyl)-ethyl]-1H-imidazole; see the inset of Figure 1A for its chemical structure; also see Bhana *et al.*, 2000) produces analgesia in humans (Filos *et al.*, 1994) and animals (Fisher *et al.*, 1991; Takano and Yaksh, 1991), possibly as a result of inhibiting glutamatergic excitatory synaptic transmission in spinal dorsal horn neurones (Sullivan *et al.*, 1992). Peripheral or central administration of  $\alpha_2$  adrenoceptor agonists, combined with local anaesthetics, extends the duration of peripheral nerve block in humans (Eisenach *et al.*, 1996; Singelyn *et al.*, 1996; Tschernko *et al.*, 1998; Madan *et al.*, 2001; Memiş *et al.*, 2004; Kanazi *et al.*, 2006) and animals

(Calasans-Maia *et al.*, 2005; Brummett *et al.*, 2008), possibly due to a constriction of local vessels by the agonists, resulting in a decrease in the clearance of the anaesthetics from the subarachnoid space (Concepcion *et al.*, 1984; Vaida *et al.*, 1986). Moreover,  $\alpha_2$  adrenoceptor agonists inhibit nerve action potential (AP) conduction and thus have a local anaesthetic effect, contributing to the enhancement of local anaesthetic effect (Gaumann *et al.*, 1992). Clonidine inhibits excitatory transmission in substantia gelatinosa (SG; lamina II of Rexed) neurones of the rat spinal cord (Pan *et al.*, 2002; Kawasaki *et al.*, 2003) and blocks AP conduction in peripheral nerves (Starke *et al.*, 1972; Gaumann *et al.*, 1992; Butterworth *et al.*, 1993); the latter action requiring a much higher concentration of clonidine than the former. It has been recently reported that dexmedetomidine as well as clonidine depresses excitatory transmission in rat SG neurones (Ishii *et al.*, 2008). Intracutaneously injected dexmedetomidine or clonidine together with lidocaine into the back of guinea-pigs enhances a degree of the local anaesthesia of lidocaine (Yoshitomi *et al.*, 2008). It is possible that, like clonidine, dexmedetomidine has an inhibitory action on AP conduction, because dexmedetomidine depresses voltage-gated  $\text{Na}^+$ -channel currents (Oda *et al.*, 2007). To our knowledge, however, whether nerve conduction is affected by dexmedetomidine has not been fully examined yet.

We have recently revealed that a variety of opioids having different chemical structures inhibit AP conduction in the frog sciatic nerve without opioid-receptor activation and that a difference in the extent of this inhibition among opioids may be attributed to a distinction in their chemical structures (Katsuki *et al.*, 2006; Mizuta *et al.*, 2008). In order to know whether dexmedetomidine affected AP conduction and if so what were the mechanisms for this action, we examined the effects of dexmedetomidine and various drugs related to adrenoceptors on a fast-conducting and  $\text{Na}^+$ -channel blocker tetrodotoxin (TTX)-sensitive compound action potential (CAP) recorded from the frog sciatic nerve by using the air-gap method. These effects were quantitatively compared with those of a well-known local anaesthetic tetracaine (Starke *et al.*, 1972; Concepcion *et al.*, 1984; see Catterall and Mackie, 2006), and also of other local anaesthetics (lidocaine, ropivacaine and cocaine) on frog sciatic nerve CAP, as reported previously (Katsuki *et al.*, 2006; Mizuta *et al.*, 2008).

## Methods

### Animals

This study was approved by the Animal Care and Use Committee of Saga University, and was

conducted in accordance with the Guiding Principles for the Care and Use of Animals in the field of Physiological Science of the Physiological Society of Japan. All efforts were made to minimize animal suffering and the number of animals used.

### *Preparation of frog sciatic nerves*

The method used for obtaining frog sciatic nerve preparation has been described previously (Katsuki *et al.*, 2006; Mizuta *et al.*, 2008). In brief, frogs (*Rana nigromaculata*; either sex) were decapitated and then pithed; thereafter the sciatic nerve was dissected from the lumbar plexus to the knee in Ringer solution. The isolated sciatic nerve was carefully desheathed under a binocular microscope and then loosely placed in five platinum wires that were glued to a Lucite plate (transparent synthetic resin), where the two ends of the nerve were tied to the wires by using threads. The plate was put on a beaker having Ringer solution in which the sciatic nerve was soaked. Throughout experiments, the Ringer solution was continuously stirred at a rate of about 350 rpm with a Teflon-covered magnetic stirrer bar in order to maintain a uniform composition of Ringer solution around the sciatic nerve. The composition of Ringer solution used was (mmol·L<sup>-1</sup>): NaCl, 115.5; KCl, 2.0; CaCl<sub>2</sub>, 1.8; Na<sub>2</sub>HPO<sub>4</sub>, 1.3; and NaH<sub>2</sub>PO<sub>4</sub>, 0.7 (pH = 7.0). Before the start of the experiment, the sciatic nerve was preincubated for at least 15 min with Ringer solution.

### *Recordings of CAPs from frog sciatic nerve fibres*

As performed previously (Katsuki *et al.*, 2006; Mizuta *et al.*, 2008), the Lucite plate having platinum wires attached with the sciatic nerve was moved from the beaker containing Ringer solution to an empty one and then CAPs were recorded in air using a preamplifier. Here, two of the platinum wires were used to record CAPs, and other two were for stimulating the sciatic nerve. The stimulation was performed at a frequency of 1 Hz with a stimulator, where rectangular pulses having 0.1 ms duration and various strengths were used. In order not to dry the sciatic nerve in air, this procedure was quickly (20 sec at the most) performed at a time interval of 2 min. When the effects of drugs on CAPs were examined, the nerve was put back into the soaking solution with drugs in between two measurements. The data were monitored on a storage oscilloscope while being recorded on a thermal array recorder having a wave form storage module and stored on magnetic tape with a PCM tape recorder for later analyses. In several cases, the data were analysed with a personal computer by using pCLAMP 8.0 software (Molecular Devices, Foster City, CA, USA).

Stimulating the sciatic nerve produced a CAP following a stimulus artefact. The peak amplitude of the CAP was measured as a difference between baseline and CAP peak level and the half-peak duration (HPD) as a time of CAP duration at a half of the peak amplitude, as previously described (Katsuki *et al.*, 2006; Mizuta *et al.*, 2008). The peak amplitude of the CAP depended on the strength of stimulus given to the sciatic nerve such that the CAP peak amplitude enhanced with an increase in stimulus strength and attained a maximal value (Katsuki *et al.*, 2006; Mizuta *et al.*, 2008). In the present study, we analysed the peak amplitude of the maximal CAP, although a further increase in stimulus strength resulted in producing a CAP which had smaller values of peak amplitude and conduction velocity (CV) than those of CAPs elicited at less stimulus strength. Such a fast-conducting CAP was completely blocked by TTX (1 µmol·L<sup>-1</sup>) in a reversible manner. Each of sciatic nerves was used only once to examine the effect of a drug on CAPs unless otherwise mentioned. A CV value was determined by using the fifth electrode as an additional stimulation site and then by measuring a change in time between stimulus artefact and the peak of CAP. All experiments were carried out at room temperature (22–27°C).

### *Data analysis*

Concentration-dependence curve for the reduction of the peak amplitude of CAP in the sciatic nerve soaked with a drug was analysed using the following Hill equation:

$$\text{CAP amplitude (\% of control)} = 100 / (1 + ([\text{Drug}] / \text{IC}_{50})^{n_H}),$$

where [Drug] is drug concentration, IC<sub>50</sub> is the concentration of drug for half-maximal inhibition and n<sub>H</sub> is the Hill coefficient.

Data are shown as mean ± SEM and statistical significance was set at *P* < 0.05 using a paired Student's *t*-test. In all cases *n* refers to the number of sciatic nerves studied.

### *Materials*

Drugs used were dexmedetomidine hydrochloride, atipamezole hydrochloride (given kindly by ORION PHARMA, Turku, Finland), yohimbine hydrochloride, (-)-phenylephrine, tetracaine hydrochloride, (±)-adrenaline (Wako, Osaka, Japan), (±)-noradrenaline (Aldrich Chem. Co., Milwaukee, WI, USA), (-)-isoprenaline hydrochloride, clonidine hydrochloride, oxymetazoline hydrochloride and tetraethylammonium (TEA) chloride (Sigma, St. Louis, MO, USA). All of drugs were directly dissolved in Ringer solution which was made up of inorganic

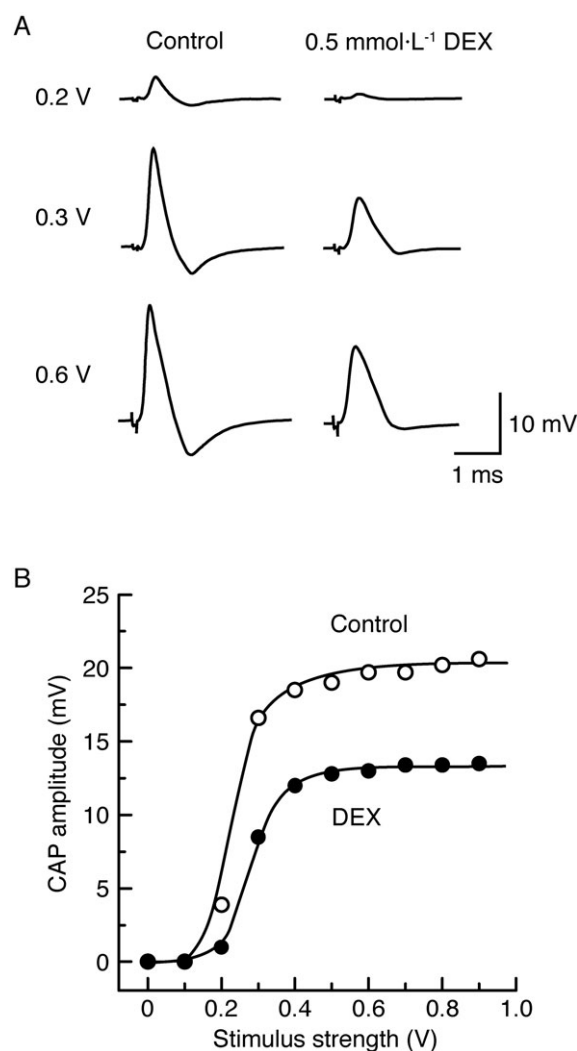
salts of extra pure grade. The pH of Ringer solution containing dexmedetomidine, (-)-phenylephrine, ( $\pm$ )-adrenaline, ( $\pm$ )-noradrenaline, (-)-isoprenaline and oxymetazoline was adjusted to 7.0 with  $1 \text{ mol}\cdot\text{L}^{-1}$ -NaOH, because it is well-known that the action of local anaesthetics on nerve conduction depends on the pH of Ringer solution used (Skou, 1954). Nomenclature of all receptors, drugs, enzymes and ion channels follows Alexander *et al.* (2009).

## Results

Effects of drugs on fast-conducting TTX-sensitive CAPs were examined in a total of 185 sciatic nerves, and when measured in some of the nerves, the CAPs had a CV of  $32 \pm 1 \text{ m}\cdot\text{s}^{-1}$  (range: 20–47  $\text{m}\cdot\text{s}^{-1}$ ;  $n = 32$ ), values comparable to those reported previously (Katsuki *et al.*, 2006; Mizuta *et al.*, 2008).

### Effects of dexmedetomidine on frog sciatic nerve CAPs

As seen in the lower traces of Figure 1A, soaking the sciatic nerve in dexmedetomidine ( $0.5 \text{ mmol}\cdot\text{L}^{-1}$ )-containing Ringer solution reversibly reduced the peak amplitude of the CAP; this reduction was accompanied by an increase in its HPD. Figure 1B demonstrates an average of the time courses of a change in CAP peak amplitude following this treatment with dexmedetomidine ( $0.5 \text{ mmol}\cdot\text{L}^{-1}$ ), relative to control, which are obtained from five sciatic nerves. The dexmedetomidine-induced reduction in CAP peak amplitude was close to a steady effect at 20 min of exposure, where the peak amplitude of CAP was  $24 \pm 8\%$  ( $P < 0.05$ ) of control ( $18 \pm 4 \text{ mV}$ ;  $n = 5$ ) and its HPD was  $142 \pm 15\%$  ( $P < 0.05$ ) of control ( $0.49 \pm 0.09 \text{ ms}$ ;  $n = 5$ ). After 30 min of exposure of the sciatic nerve to dexmedetomidine-free solution, the CAP amplitude recovered to control level, as shown in Figure 1. Figure 2 demonstrates the effect of dexmedetomidine ( $0.5 \text{ mmol}\cdot\text{L}^{-1}$ ) on CAPs elicited at various stimulus strengths given to the sciatic nerve. The inhibitory effect of dexmedetomidine was seen for CAPs evoked at a maximal stimulus strength while a threshold to elicit CAPs was not changed by dexmedetomidine. Such a result was obtained in six other nerves. Figure 3A demonstrates the time courses of changes in CAP peak amplitude after treatment with dexmedetomidine at various concentrations ranging from  $0.01$  to  $1 \text{ mmol}\cdot\text{L}^{-1}$ . The rate of the reduction of CAP peak amplitude produced by dexmedetomidine was concentration-dependent as was the magnitude of the reduction at 20 min (Figure 3Ba). The corresponding concentration-

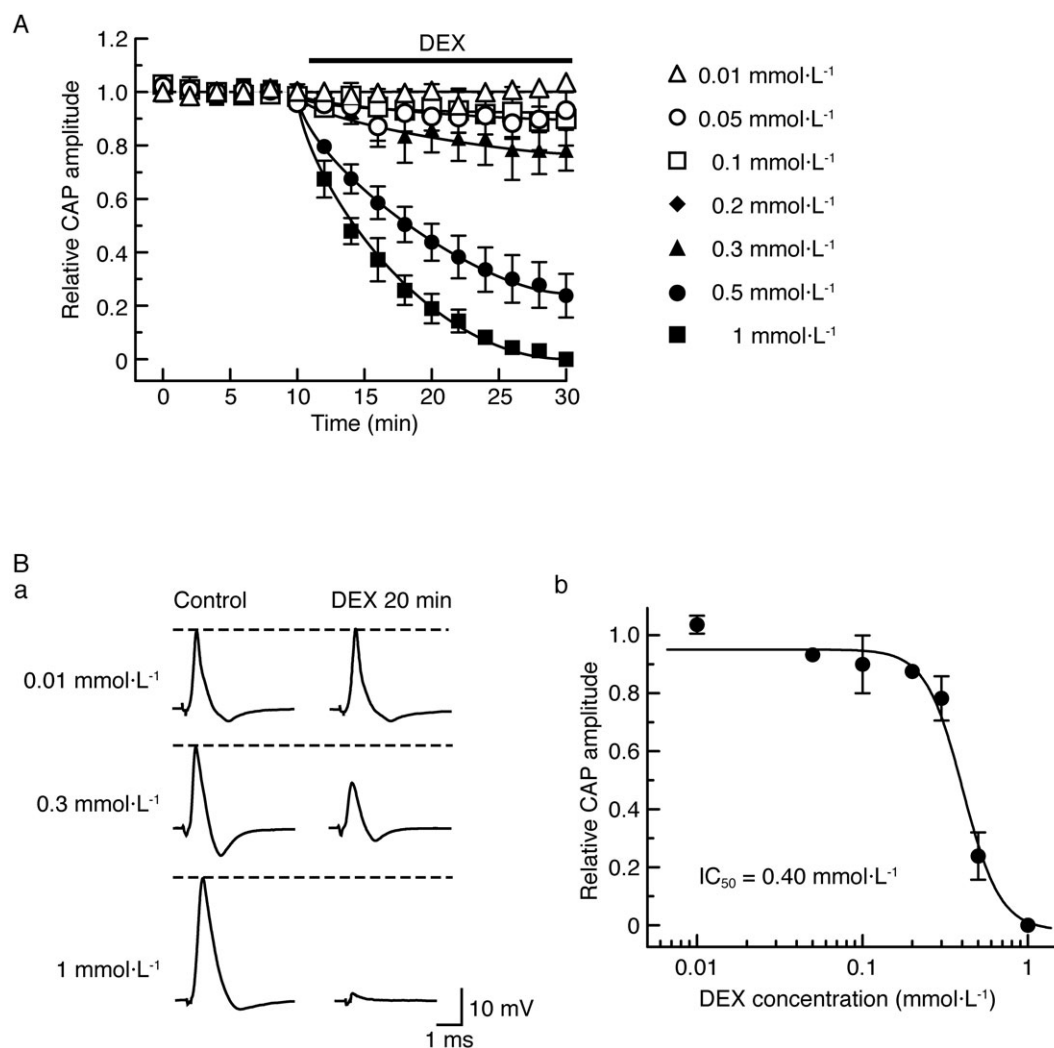


**Figure 2**

The reduction in compound action potential (CAP) peak amplitude by dexmedetomidine (DEX;  $0.5 \text{ mmol}\cdot\text{L}^{-1}$ ) is seen without a change in a threshold to elicit CAPs and with a decrease in maximal responses. (A) Recordings of CAPs elicited at 0.2, 0.3 and 0.6 V in the control (left) and after the action of dexmedetomidine for a period of 20 min (right). (B) The peak amplitudes of CAP before and after dexmedetomidine are plotted against stimulus strength used to elicit the CAP. The solid lines were drawn by eye.

response curve (Figure 3Bb) yielded an  $\text{IC}_{50}$  value of  $0.40 \text{ mmol}\cdot\text{L}^{-1}$  with a  $n_{\text{H}}$  of 3.9.

As dexmedetomidine exhibits a high affinity for  $\alpha_2$  adrenoceptors (Bhana *et al.*, 2000), we next investigated whether this effect of dexmedetomidine was mediated by  $\alpha_2$  adrenoceptors. Figure 4 demonstrates how the effect of dexmedetomidine ( $0.5 \text{ mmol}\cdot\text{L}^{-1}$ ) on sciatic nerve CAPs was affected by two  $\alpha_2$ -adrenoceptor antagonists, yohimbine and atipamezole (Coughlan *et al.*, 1992; Sjöholm *et al.*, 1992; Sullivan *et al.*, 1992; see Virtanen, 1989; MacDonald *et al.*, 1997; their chemical structures are



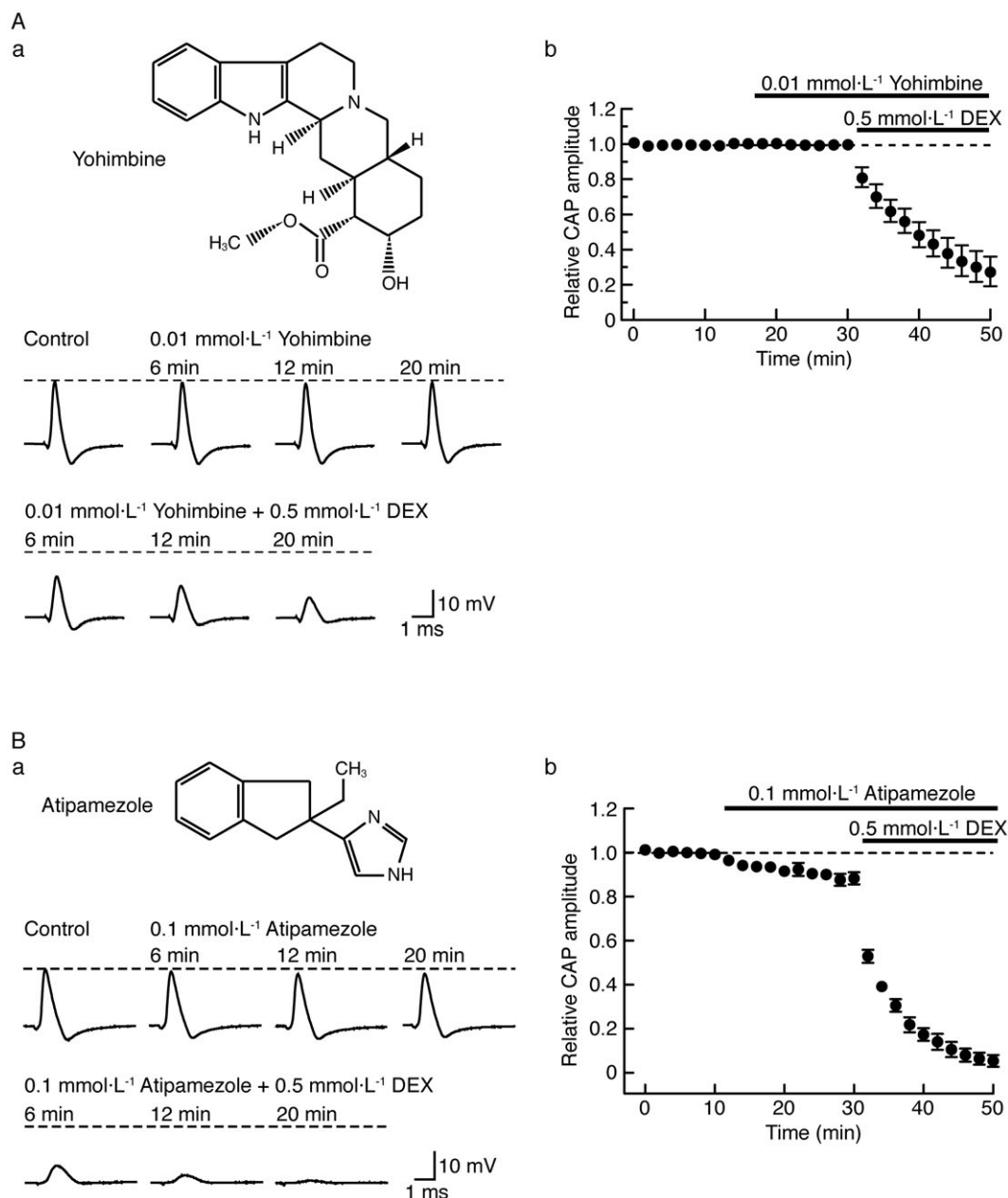
### Figure 3

Compound action potential (CAP) peak amplitude is reduced by dexmedetomidine (DEX) in a concentration-dependent manner. (A) Comparison in average time course among CAP peak amplitude reductions produced by dexmedetomidine at 0.01–1 mmol·L<sup>-1</sup>, obtained from 23 sciatic nerves. The solid lines were drawn by eye. (B) Concentration dependence for CAP peak amplitude reduction by dexmedetomidine. (Ba) Recordings of CAPs in the control (left) and after 20 min of exposure to dexmedetomidine at 0.01, 0.3 and 1 mmol·L<sup>-1</sup> (right); these were obtained from different sciatic nerves. (Bb) The peak amplitude of CAP recorded from sciatic nerve fibres treated with dexmedetomidine at various concentrations for 20 min, relative to control, was plotted against dexmedetomidine concentration. Each of the data points was obtained from 3–5 sciatic nerves. The concentration-response curve was drawn according to the Hill equation (IC<sub>50</sub> = 0.40 mmol·L<sup>-1</sup>, n<sub>H</sub> = 3.9).

shown in the insets of Figure 4Aa and Ba respectively). Pretreatment of sciatic nerves with yohimbine (20 min; 0.01 mmol·L<sup>-1</sup>, a concentration enough to antagonize dexmedetomidine-induced membrane hyperpolarization mediated by  $\alpha_2$  adrenoceptors in rat hypothalamic neurones; see Shirasaka *et al.*, 2007), did not affect CAPs [peak amplitude, 100 ± 2% (*n* = 4; *P* > 0.05) of control; Figure 4A]. Adding dexmedetomidine (0.5 mmol·L<sup>-1</sup>) to yohimbine reduced peak amplitude to 28 ± 8% (*P* < 0.05) of that (22 ± 1 mV; *n* = 4) with yohimbine alone (Figure 4Aa and Ab). This value was not significantly different from that (24 ±

8%; *n* = 5; see above; *P* > 0.05) obtained by treatment with dexmedetomidine alone.

Atipamezole was used at 0.1 mmol·L<sup>-1</sup>, a concentration enough to antagonize dexmedetomidine-induced hypnotic responses, mediated by  $\alpha_2$  adrenoceptors in the rat locus coeruleus (Correa-Sales *et al.*, 1992). As noted from the traces in Figure 4Ba, pretreatment with this drug itself for 20 min reduced the peak amplitude of CAP, but only slightly to 88 ± 3% (*P* < 0.05) of control (21 ± 2 mV; *n* = 5). Adding dexmedetomidine (0.5 mmol·L<sup>-1</sup>) to atipamezole markedly inhibited the CAP, as summarized in Figure 4Bb. The peak amplitude after

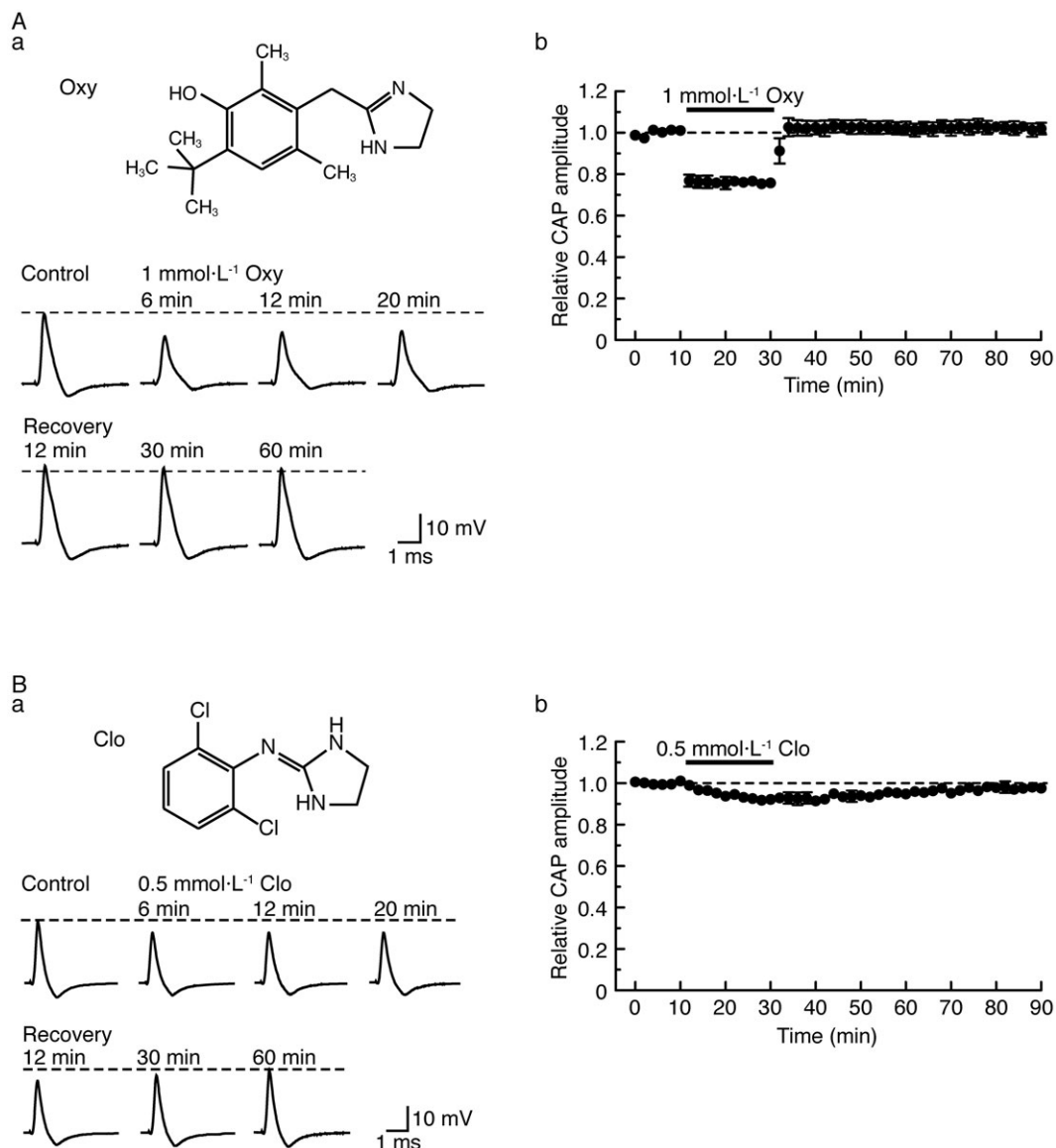


**Figure 4**

Dexmedetomidine (DEX; 0.5 mmol·L<sup>-1</sup>)-induced reduction in compound action potential (CAP) peak amplitude is resistant to  $\alpha_2$ -adrenoceptor antagonists, yohimbine (0.01 mmol·L<sup>-1</sup>) and atipamezole (0.1 mmol·L<sup>-1</sup>). (A, B) Effects of DEX on CAP peak amplitude in the presence of  $\alpha_2$  antagonists (A: yohimbine; B: atipamezole). (Aa, Ba) Recordings of CAPs in the control, after 6, 12 and 20 min of exposure to  $\alpha_2$  antagonists (Aa: yohimbine; Ba: atipamezole) and thereafter 6, 12 and 20 min in the presence of DEX together with  $\alpha_2$  antagonists (Aa: yohimbine; Ba: atipamezole). Upper insets in (Aa) and (Ba) show the chemical structures of yohimbine and atipamezole (see Virtanen, 1989; Westfall and Westfall, 2006), respectively. (Ab, Bb) Average time courses of changes in CAP peak amplitudes following treatment with  $\alpha_2$  antagonists (Ab: yohimbine; Bb: atipamezole) and with both DEX and  $\alpha_2$  antagonists (Ab: yohimbine; Bb: atipamezole), relative to that before drug treatment, obtained from four (Ab) or five (Bb) sciatic nerves. Note that atipamezole (0.1 mmol·L<sup>-1</sup>) itself reduces CAP peak amplitudes.

20 min with atipamezole and dexmedetomidine was  $6 \pm 3\%$  ( $P < 0.05$ ) of that ( $19 \pm 2$  mV;  $n = 5$ ) just before the co-treatment and was significantly less than that ( $24 \pm 8\%$ ;  $n = 5$ ; see above;  $P < 0.05$ ) obtained with dexmedetomidine only. This differ-

ence may be due to an interaction between the effects of dexmedetomidine and atipamezole. At a higher concentration (0.2 mmol·L<sup>-1</sup>), atipamezole by itself reduced CAP peak amplitude to  $40 \pm 5\%$  ( $P < 0.05$ ) of control ( $21 \pm 2$  mV;  $n = 5$ ).



**Figure 5**

Other  $\alpha_2$ -adrenoceptor agonists, oxymetazoline (Oxy; 1 mmol·L<sup>-1</sup>) and clonidine (Clo; 0.5 mmol·L<sup>-1</sup>), also reversibly reduce compound action potential (CAP) peak amplitudes. (A, B) Effects of  $\alpha_2$  agonists (A: Oxy; B: Clo) on CAP peak amplitudes. (Aa, Ba) Recordings of CAPs in the control, after 6, 12 and 20 min of exposure to  $\alpha_2$  adrenoceptor agonists (A: Oxy; B: Clo) and thereafter 12, 30 and 60 min in the absence of  $\alpha_2$  adrenoceptor agonists (A: Oxy; B: Clo). Upper insets in (Aa) and (Ba) show the chemical structures of oxymetazoline and clonidine, respectively (see Westfall and Westfall, 2006). (Ab, Bb) Average time courses of changes in CAP peak amplitudes following exposure to  $\alpha_2$  adrenoceptor agonists (Ab: Oxy; Bb: Clo) for 20 min, relative to that before exposure, obtained from four (Ab) or five (Bb) sciatic nerves.

### Effects of various adrenoceptor agonists on frog sciatic nerve CAPs

Next, we investigated whether CAPs are affected by other  $\alpha_2$ -adrenoceptor agonists, oxymetazoline (more selective for  $\alpha_{2A}$  than  $\alpha_{2B}$  and  $\alpha_{2C}$  adrenoceptors; see Bylund *et al.*, 1994; MacDonald *et al.*, 1997) and clonidine (their chemical structures are shown in the insets of Figure 5Aa and Ba, respectively). As seen with dexmedetomidine, oxymetazoline (1 mmol·L<sup>-1</sup>) reduced CAP peak amplitude in a

reversible manner, as shown by the traces in Figure 5Aa. Figure 5Ab summarizes the time courses of a change in CAP peak amplitude following treatment with oxymetazoline (1 mmol·L<sup>-1</sup>). More rapidly than dexmedetomidine, oxymetazoline (1 mmol·L<sup>-1</sup>) reduced CAP peak amplitude to a steady state of  $76 \pm 2\%$  ( $P < 0.05$ ) of control ( $20 \pm 2$  mV;  $n = 4$ ) with a slight tendency to increase the HPD of CAP [ $106 \pm 9\%$  ( $P > 0.05$ ) of control ( $0.45 \pm 0.06$  ms;  $n = 4$ )].

As there was a remarkable difference between the time taken for dexmedetomidine- and oxymetazoline-induced CAP inhibition to attain a steady state (see Figures 1B and 5Ab), we further examined the effects of both dexmedetomidine ( $0.5 \text{ mmol}\cdot\text{L}^{-1}$ ) and oxymetazoline ( $1 \text{ mmol}\cdot\text{L}^{-1}$ ) on CAPs in the same sciatic nerve. The CAP inhibition produced by oxymetazoline attained a steady level at 2 min following drug treatment; the peak amplitude of CAP at this time was the same ( $101 \pm 2\%$ ;  $n = 3$ ) as that at 20 min (100%). On the other hand, CAP inhibition produced by dexmedetomidine was clearly dependent on the time of exposure. Thus, CAP peak amplitudes at 2, 6 and 16 min following drug treatment were, respectively,  $259 \pm 36$ ,  $224 \pm 35$  and  $118 \pm 3\%$  ( $n = 3$ ) of that at 20 min (100%). Figure 6A and B show that the effects of oxymetazoline on CAPs were concentration-dependent over a wide range ( $0.02\text{--}5 \text{ mmol}\cdot\text{L}^{-1}$ ). At  $2 \text{ mmol}\cdot\text{L}^{-1}$ , oxymetazoline also increased the HPD of CAP [ $158 \pm 18\%$  ( $n = 4$ ;  $P < 0.05$ ) of control]. The full concentration-response curve is shown in Figure 6Bb and provided an  $\text{IC}_{50}$  value of  $1.5 \text{ mmol}\cdot\text{L}^{-1}$  with a  $n_{\text{H}}$  of 1.5.

Although clonidine ( $0.5 \text{ mmol}\cdot\text{L}^{-1}$ ) also reduced CAP peak amplitude in a reversible manner (Figure 5B), this effect of clonidine was less marked than that of dexmedetomidine or oxymetazoline. At a higher concentration ( $2 \text{ mmol}\cdot\text{L}^{-1}$ ), clonidine was no more effective than at  $0.5 \text{ mmol}\cdot\text{L}^{-1}$  (Figure 6Ca) and the corresponding concentration-response curve for clonidine on CAP amplitude (Figure 6Cb) showed a maximum reduction by clonidine of about 20%.

Yohimbine ( $0.01 \text{ mmol}\cdot\text{L}^{-1}$ ) did not affect the inhibitory actions of oxymetazoline ( $1 \text{ mmol}\cdot\text{L}^{-1}$ ) and clonidine ( $0.5 \text{ mmol}\cdot\text{L}^{-1}$ ) on CAPs (peak amplitudes at 20 min: oxymetazoline only,  $76 \pm 2\%$ ;  $n = 4$ ; yohimbine and oxymetazoline,  $79 \pm 7\%$ ,  $n = 3$ ;  $P > 0.05$ : clonidine only,  $93 \pm 2\%$ ;  $n = 9$ ; yohimbine and clonidine,  $89 \pm 5\%$ ,  $n = 3$ ;  $P > 0.05$ ).

We next examined the effects of various adrenoceptor agonists, ( $\pm$ )-adrenaline, ( $\pm$ )-noradrenaline,  $\alpha_1$ -adrenoceptor agonist (-)-phenylephrine and  $\beta$ -adrenoceptor agonist (-)-isoprenaline (their chemical structures are shown in the insets of Figure 7Aa, Ba, Ca and Da respectively) on CAPs. As seen from the traces in Figure 7Aa, Ba, Ca and Da, ( $\pm$ )-adrenaline, ( $\pm$ )-noradrenaline, (-)-phenylephrine and (-)-isoprenaline (each  $1 \text{ mmol}\cdot\text{L}^{-1}$ ) did not affect CAPs. Figure 7Ab, Bb, Cb and Db summarize the time courses of changes in CAP peak amplitudes following treatment with these agonists and in Figure 7E the mean values of CAP peak amplitudes after 20 min treatment all demonstrate no effects of the adrenoceptor agonists ( $1 \text{ mmol}\cdot\text{L}^{-1}$ ).

### Effect of tetracaine on frog sciatic nerve CAPs

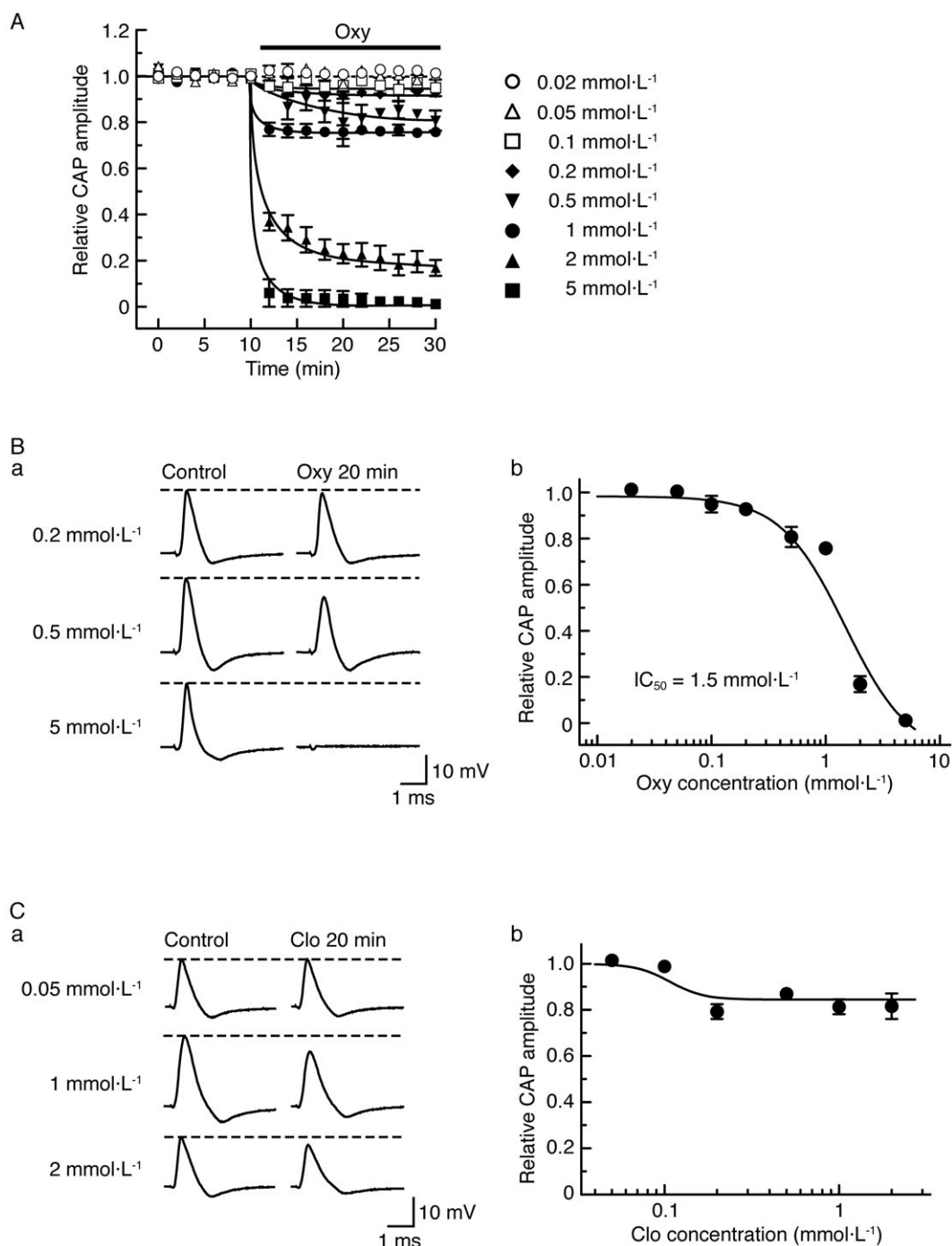
In order to compare the inhibitory effect of dexmedetomidine on CAPs with that of local anaesthetics, we next examined the effect of a well-known local anaesthetic tetracaine on CAPs. Tetracaine at a concentration of  $0.02 \text{ mmol}\cdot\text{L}^{-1}$  reversibly reduced CAP peak amplitude, as seen in Figure 8Aa and summarized in Figure 8Ab. The maximal effect of tetracaine ( $0.02 \text{ mmol}\cdot\text{L}^{-1}$ ) on CAP amplitude was reached within 20 min of exposure, reducing peak amplitude to  $27 \pm 7\%$  ( $P < 0.05$ ) of control ( $20 \pm 2 \text{ mV}$ ;  $n = 7$ ) with an increase in the HPD of CAP [ $136 \pm 11\%$  ( $P < 0.05$ ) of control ( $0.63 \pm 0.06 \text{ ms}$ ;  $n = 7$ )]. The concentration-dependence of the effects of tetracaine ( $0.0005\text{--}0.05 \text{ mmol}\cdot\text{L}^{-1}$ ) on CAPs is shown in Figure 8B and C. The rate of the reduction of CAP peak amplitude produced by tetracaine was concentration-dependent as was the magnitude of the reduction at 20 min. Analysis of the concentration-response curve provided an  $\text{IC}_{50}$  value for tetracaine of  $0.014 \text{ mmol}\cdot\text{L}^{-1}$  with a  $n_{\text{H}}$  of 1.4.

In order to define the channels affected by dexmedetomidine, we examined how the inhibitory action of dexmedetomidine ( $0.5 \text{ mmol}\cdot\text{L}^{-1}$ ) on CAPs was altered by treating the nerve trunk with tetracaine ( $0.01 \text{ mmol}\cdot\text{L}^{-1}$ ; which is well-known to inhibit voltage-gated  $\text{Na}^+$  channels; Bräu *et al.*, 1998) or an inhibitor of delayed-rectifier  $\text{K}^+$ -channels TEA ( $1 \text{ mmol}\cdot\text{L}^{-1}$ ) for 20 min. Tetracaine reduced CAP peak amplitude [ $62 \pm 5\%$  ( $n = 4$ ;  $P < 0.05$ ) of control] and increased the HPD of CAP [ $156 \pm 12\%$  ( $n = 4$ ;  $P < 0.05$ ) of control]; adding dexmedetomidine completely blocked the CAPs. TEA increased the HPD of CAP [ $120 \pm 4\%$  ( $n = 4$ ;  $P < 0.05$ ) of control] without a change in CAP peak amplitude [ $100 \pm 1\%$  ( $n = 4$ ;  $P > 0.05$ ) of control; see Mizuta *et al.*, 2008]; after adding dexmedetomidine, the HPD of CAP was increased further ( $186 \pm 12\%$ ;  $n = 3$ ;  $P < 0.05$ ) and the CAP peak amplitude markedly reduced ( $6 \pm 2\%$ ;  $n = 4$ ;  $P < 0.05$ ).

## Discussion

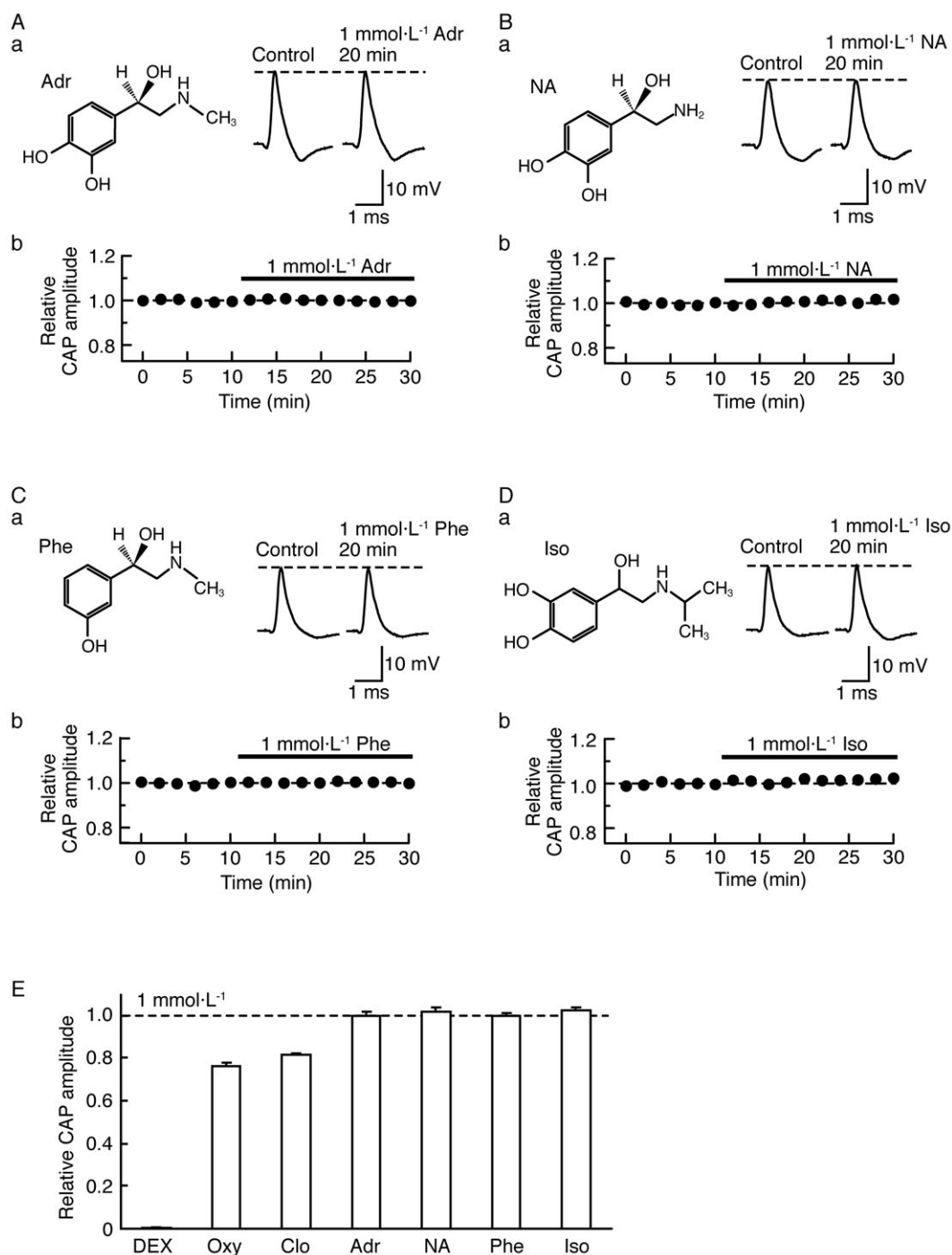
The present study demonstrated that the  $\alpha_2$ -adrenoceptor agonist dexmedetomidine reversibly and concentration-dependently reduced CAP peak amplitude with an  $\text{IC}_{50}$  value of  $0.40 \text{ mmol}\cdot\text{L}^{-1}$  in the frog sciatic nerve. A reduction in amplitude was produced by other  $\alpha_2$ -adrenoceptor agonists, oxymetazoline ( $\text{IC}_{50} = 1.5 \text{ mmol}\cdot\text{L}^{-1}$ ) and clonidine (by 20% at  $2 \text{ mmol}\cdot\text{L}^{-1}$ ). Inhibition of CAPs by clonidine has been reported in the frog and rat sciatic nerve (Starke *et al.*, 1972; Butterworth *et al.*, 1993), although their results are quantitatively different





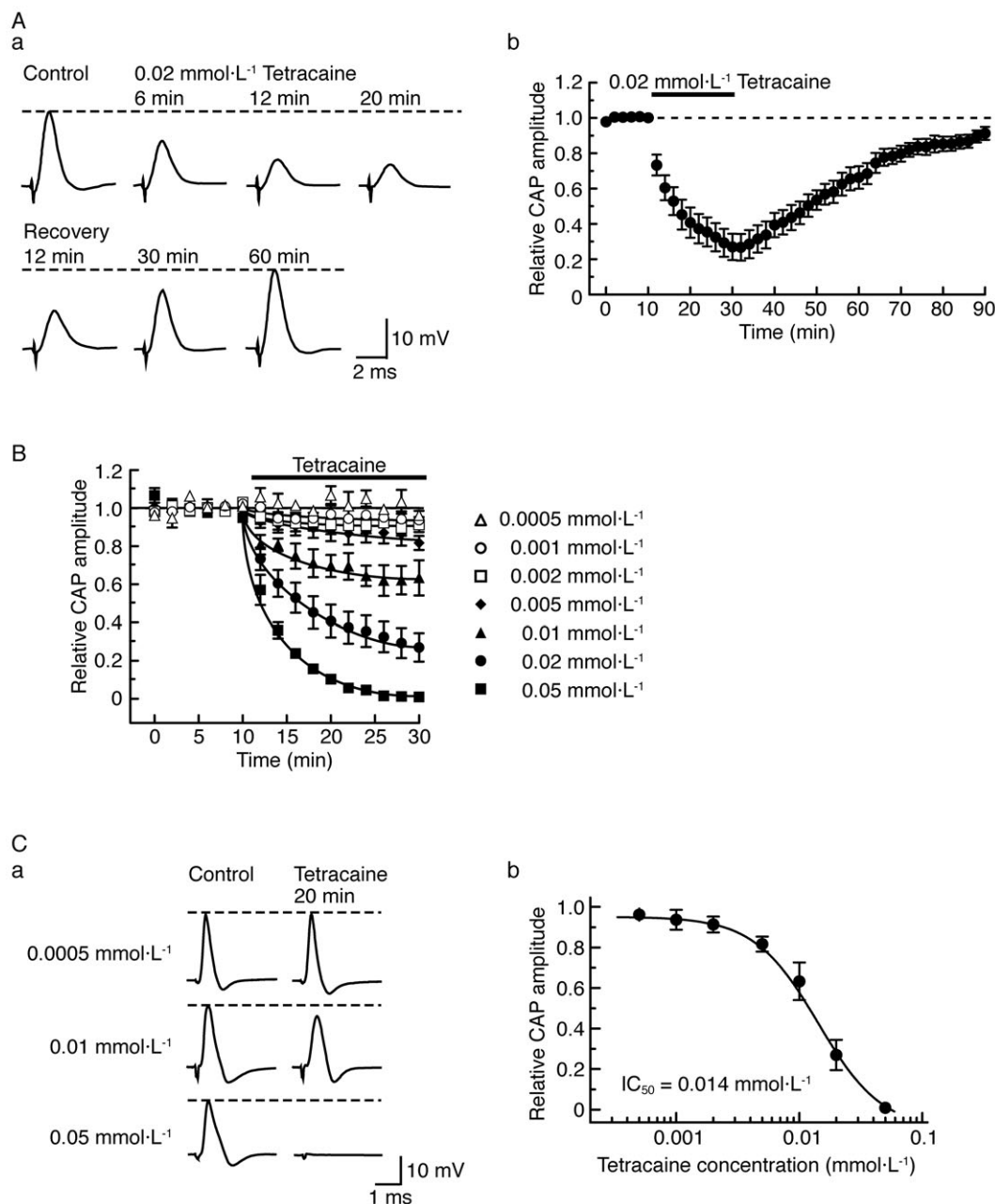
**Figure 6**

$\alpha_2$ -Adrenoceptor agonists, oxymetazoline (Oxy) and clonidine (Clo), reduce compound action potential (CAP) peak amplitudes in a concentration-dependent manner. (A) Comparison in average time course among CAP peak amplitude reductions produced by oxymetazoline at 0.02–5 mmol·L<sup>-1</sup>, obtained from 25 sciatic nerves. The solid lines were drawn by eye. (B) Concentration dependence for the effect of oxymetazoline on CAPs. (Ba) Recordings of CAPs in the control (left) and after 20 min of exposure to oxymetazoline at 0.2, 0.5 and 5 mmol·L<sup>-1</sup> (right); these were obtained from different sciatic nerves. (Bb) The peak amplitude of CAP recorded from sciatic nerve fibres treated with oxymetazoline at various concentrations for 20 min, relative to control, which was plotted against oxymetazoline concentration. Each of the data points was obtained from 3–4 sciatic nerves. The concentration–response curve in (Bb) was drawn according to the Hill equation (IC<sub>50</sub> = 1.5 mmol·L<sup>-1</sup>, n<sub>H</sub> = 1.5). (C) Concentration dependence for CAP peak amplitude reduction by clonidine. (Ca) Recordings of CAPs in the control (left) and after 20 min of exposure to clonidine at 0.05, 1 and 2 mmol·L<sup>-1</sup> (right); these were obtained from different sciatic nerves. (Cb) The peak amplitude of CAP recorded from sciatic nerve fibres treated with clonidine at various concentrations for 20 min, relative to control, was plotted against clonidine concentration. Each of the data points was obtained from 3–5 sciatic nerves.



**Figure 7**

Effects of various adrenoceptor agonists on compound action potentials (CAPs). (Aa, Ba, Ca, Da) Recordings of CAPs in the control (left) and after 20 min of exposure to ( $\pm$ )-adrenaline (Adr; Aa), ( $\pm$ )-noradrenaline (NA; Ba),  $\alpha_1$ -adrenoceptor agonist (-)-phenylephrine (Phe; Ca) and  $\beta$ -adrenoceptor agonist (-)-isoprenaline (Iso; Da) at 1 mmol·L<sup>-1</sup> (right); these were obtained from different sciatic nerves. Insets in (Aa), (Ba), (Ca) and (Da) show the chemical structures of adrenaline, noradrenaline, phenylephrine and isoprenaline, respectively (see Westfall and Westfall, 2006). (Ab, Bb, Cb, Db) Average time courses of changes in CAP peak amplitudes following treatment with adrenaline (Ab;  $n = 3$ ), noradrenaline (Bb;  $n = 3$ ), phenylephrine (Cb;  $n = 3$ ) or isoprenaline (Db;  $n = 3$ ), relative to that before drug treatment. (E) CAP amplitude (Relative CAP amplitude) after 20 min of exposure to adrenoceptor agonists [dexmedetomidine (DEX), Oxy, Clo, Adr, NA, Phe and Iso; each 1 mmol·L<sup>-1</sup>], relative to control. The relative CAP amplitudes under the actions of DEX, oxymetazoline and clonidine were taken from Figures 3Bb, 6Bb and Cb, respectively.



**Figure 8**

Effect of tetracaine on compound action potentials (CAPs) recorded from frog sciatic nerve fibres. (A) Tetracaine at a concentration of 0.02 mmol·L<sup>-1</sup> reversibly reduces CAP peak amplitudes. (Aa) Recordings of CAPs in the control, after 6, 12 and 20 min of exposure to tetracaine and thereafter 12, 30 and 60 min in the absence of tetracaine. (Ab) Average time course of changes in CAP peak amplitudes following exposure to tetracaine for 20 min, relative to that before treatment, obtained from seven sciatic nerves. (B) Comparison in average time course among CAP peak amplitude reductions produced by tetracaine at 0.0005–0.05 mmol·L<sup>-1</sup>, obtained from 28 sciatic nerves. The solid lines were drawn by eye. (C) Concentration dependence for CAP peak amplitude reduction by tetracaine. (Ca) Recordings of CAPs in the control (left) and after 20 min of exposure to tetracaine at 0.0005, 0.01 and 0.05 mmol·L<sup>-1</sup> (right); these were obtained from different sciatic nerves. (Cb) The peak amplitude of CAP recorded from sciatic nerve fibres treated with tetracaine at various concentrations for 20 min, relative to control, was plotted against tetracaine concentration. Each of the data points was obtained from 3–7 sciatic nerves. The concentration-response curve was drawn according to the Hill equation (IC<sub>50</sub> = 0.014 mmol·L<sup>-1</sup>, n<sub>H</sub> = 1.4).

from ours (see below). The higher potency of dexmedetomidine than clonidine may indicate that the CAP inhibition is mediated by  $\alpha_2$  adrenoceptors, because dexmedetomidine is about eightfold more

potent than clonidine in binding to the  $\alpha_2$  adrenoceptors of rat brain membrane preparations (Virtanen *et al.*, 1988; Savola and Virtanen, 1991). Dexmedetomidine acts as a full agonist at  $\alpha_2$  adreno-

ceptors while clonidine is a partial agonist in diminishing halothane anaesthetic requirement in rats (Maze *et al.*, 1987; Segal *et al.*, 1988), observations similar to ours on frog CAP (see Figures 3Bb and 6Cb). The activity of dexmedetomidine ( $0.5 \text{ mmol}\cdot\text{L}^{-1}$ ) in the present study, however, was resistant to an  $\alpha_2$ -adrenoceptor antagonist yohimbine ( $0.01 \text{ mmol}\cdot\text{L}^{-1}$ ). The activities of oxymetazoline ( $1 \text{ mmol}\cdot\text{L}^{-1}$ ) and clonidine ( $0.5 \text{ mmol}\cdot\text{L}^{-1}$ ) were also unaffected by yohimbine. Although another  $\alpha_2$ -adrenoceptor antagonist atipamezole ( $0.1 \text{ mmol}\cdot\text{L}^{-1}$ ) itself slightly reduced CAP amplitude, the dexmedetomidine-induced CAP inhibition was almost unaffected by atipamezole. Atipamezole-induced CAP inhibition was concentration-dependent in a range of  $0.1\text{--}0.2 \text{ mmol}\cdot\text{L}^{-1}$ . These results indicate no involvement of  $\alpha_2$  adrenoceptors. Unlike  $\alpha_2$  adrenoceptor-related drugs, ( $\pm$ )-adrenaline, ( $\pm$ )-noradrenaline,  $\alpha_1$ -adrenoceptor agonist (-)-phenylephrine and  $\beta$ -adrenoceptor agonist (-)-isoprenaline at  $1 \text{ mmol}\cdot\text{L}^{-1}$  had no effect on CAPs. Thus, the dexmedetomidine effect was not mediated by adrenoceptors, in general.

In our study, the clonidine-induced reduction of CAP amplitude was concentration-dependent in a range of  $0.05\text{--}0.2 \text{ mmol}\cdot\text{L}^{-1}$  with a maximal value of 20%; a further reduction was not seen at  $> 0.2 \text{ mmol}\cdot\text{L}^{-1}$  (Figure 6C). In contrast to this result, Starke *et al.* (1972) have reported a concentration-dependent inhibition of frog sciatic nerve CAP in a very narrow concentration range around  $0.2 \text{ mmol}\cdot\text{L}^{-1}$  with 80% inhibition at  $0.3 \text{ mmol}\cdot\text{L}^{-1}$ . The reason for the discrepancy between these results is unknown. The CAPs of A $\alpha$  and C fibres in the rat sciatic nerve were inhibited by clonidine with different  $\text{IC}_{50}$  values ( $2.0$  and  $0.45 \text{ mmol}\cdot\text{L}^{-1}$  respectively; Butterworth *et al.*, 1993). The concentration dependency for clonidine-mediated CAP inhibition in the present study may be due to the presence of clonidine-sensitive and -insensitive fast-conducting (possibly A-type) fibres in the frog sciatic nerve. This issue remains to be further examined.

It may be of interest to note a difference in onset and offset time between CAP peak amplitude reductions produced by dexmedetomidine and oxymetazoline (see Figures 1B and 5Ab). Although dexmedetomidine was more effective than oxymetazoline in reducing CAP amplitude, oxymetazoline had shorter onset and offset times than dexmedetomidine. This may be due to a difference between their chemical structures, in that oxymetazoline has more methyl groups and is thus more lipophilic than dexmedetomidine (see Figures 1A and 5Aa). The  $\alpha_2$  antagonist atipamezole appeared to be more effective in inhibiting CAP than dexmedetomidine and oxymetazoline. A chemical struc-

ture related to  $\alpha_2$ -adrenoceptor drugs, particularly dexmedetomidine and atipamezole, may play an important role in producing the CAP inhibition.

Although dexmedetomidine inhibits hyperpolarization-activated  $\text{K}^+$  channels and thus blocks nerve conduction by activating  $\alpha_2$  adrenoceptors (Dalle *et al.*, 2001; Shirasaka *et al.*, 2007), this is unlikely in the present study. The dexmedetomidine-induced CAP inhibition in our study was resistant to  $\alpha_2$ -adrenoceptor antagonists and was not accompanied by a change in the threshold to elicit CAPs (see Figure 2B) which may be expected when hyperpolarization-activated  $\text{K}^+$  channels are inhibited (Dalle *et al.*, 2001). Our preparation is the dissected sciatic nerve which lacked the neuronal cell body and the neuronal terminals. The inhibitory effect on nerve conduction is thus less likely to be related to G-protein coupled membrane receptors such as  $\alpha_2$  adrenoceptors.

The dexmedetomidine action in the present study would be due to an inhibition of TTX-sensitive voltage-gated  $\text{Na}^+$  channels and/or TEA-sensitive (delayed-rectifier)  $\text{K}^+$  channels which are involved in producing frog CAPs (Mizuta *et al.*, 2008). In support of this idea, Oda *et al.* (2007) have reported an inhibition by dexmedetomidine of voltage-gated  $\text{Na}^+$  channels in rat dorsal root ganglia (DRG) neurones in a manner resistant to yohimbine, although this type of  $\text{Na}^+$  channels is resistant to TTX. Dexmedetomidine acting on rat DRG neurones had an  $\text{IC}_{50}$  value of  $0.058 \text{ mmol}\cdot\text{L}^{-1}$ , about 10-fold smaller than that ( $0.40 \text{ mmol}\cdot\text{L}^{-1}$ ) we found for inhibition of frog CAPs. These values were  $>100$ -fold larger than that ( $0.62 \mu\text{mol}\cdot\text{L}^{-1}$ ) in producing membrane hyperpolarization by activating  $\alpha_2$  adrenoceptors in rat SG neurones (Ishii *et al.*, 2008). The rat TTX-resistant  $\text{Na}^+$  channel was also inhibited by clonidine, yohimbine and lidocaine ( $\text{IC}_{50}$ :  $0.26$ ,  $0.0026$  and  $0.073 \text{ mmol}\cdot\text{L}^{-1}$ , respectively; Oda *et al.*, 2007). Dessaint *et al.* (2004) have reported that yohimbine inhibited not only TTX-resistant  $\text{Na}^+$  channels ( $\text{IC}_{50} = 0.078 \text{ mmol}\cdot\text{L}^{-1}$ ) but also TTX-sensitive  $\text{Na}^+$  channels ( $\text{Na}_v1.2$ ;  $\text{IC}_{50} = 0.047 \text{ mmol}\cdot\text{L}^{-1}$ ; by about 10% at  $0.01 \text{ mmol}\cdot\text{L}^{-1}$ ) in rat DRG neurones. On the contrary, frog CAPs were not inhibited by yohimbine ( $0.01 \text{ mmol}\cdot\text{L}^{-1}$ ) and were more sensitive to dexmedetomidine than lidocaine by about twofold (see below). Very recently, it has been found that, in NG108-15 neuronal cells the delayed-rectifier  $\text{K}^+$ -channel current amplitudes are reduced by dexmedetomidine with an  $\text{IC}_{50}$  value of  $0.0046 \text{ mmol}\cdot\text{L}^{-1}$  and TTX-sensitive  $\text{Na}^+$ -channel current amplitudes are attenuated by about 20% by dexmedetomidine ( $0.01 \text{ mmol}\cdot\text{L}^{-1}$ ) in a manner insensitive to yohimbine (Chen *et al.*, 2009). These differences in potency among drugs

may be due to a distinction in either animal species or Na<sup>+</sup>-channel types. When dexmedetomidine acts on TTX-sensitive Na<sup>+</sup> channels and delayed-rectifier K<sup>+</sup> channels in the frog sciatic nerve, the binding sites of dexmedetomidine in the channels appear to be different from those of tetracaine and TEA, because these drugs do not prevent reduction of CAP peak amplitude and increase of HPD, both of which are produced by dexmedetomidine.

Dexmedetomidine exhibits an affinity for not only  $\alpha_2$  adrenoceptors but also imidazoline receptors (see Savola and Savola, 1996; Khan *et al.*, 1999). Dahmani *et al.* (2008) have recently reported that dexmedetomidine increased the extent of phosphorylated extracellular signal-regulated protein kinase without  $\alpha_2$ -adrenoceptor activation in the rat hippocampus, possibly by activating imidazoline I<sub>1</sub> receptors. On the other hand, the reduction of halothane anaesthetic requirement produced by dexmedetomidine in rats was not mediated by imidazoline receptors and was due to  $\alpha_2$ -adrenoceptor activation (Kagawa *et al.*, 1997). Clonidine, which exhibits a high affinity for imidazoline I<sub>1</sub> receptors (see Farsang and Kapocsi, 1999), was less potency in inhibiting frog CAP. Atipamezole does not activate imidazoline I<sub>2</sub> receptors (Diaz *et al.*, 1997) but was more effective in inhibiting CAPs than  $\alpha_2$  adrenoceptor agonists. Thus, the dexmedetomidine-mediated inhibition of frog CAPs appeared not to be mediated by imidazoline receptors.

### *Comparison between dexmedetomidine and local anaesthetics in reducing CAP peak amplitudes*

Frog CAP peak amplitude was reversibly and concentration-dependently reduced by tetracaine with an IC<sub>50</sub> of 0.014 mmol·L<sup>-1</sup> (Figure 8C). This value is not so different from that (0.0063 mmol·L<sup>-1</sup>) of frog sciatic nerve fibres, reported previously (Starke *et al.*, 1972), and also from that (0.009 mmol·L<sup>-1</sup>) of rabbit A nerve fibres (Gissen *et al.*, 1980). This value of tetracaine was about 30-fold smaller than that of dexmedetomidine (IC<sub>50</sub> = 0.40 mmol·L<sup>-1</sup>). When compared with the actions of other local anaesthetics, as reported previously (Katsuki *et al.*, 2006; Mizuta *et al.*, 2008), this dexmedetomidine action was similar to that of ropivacaine (IC<sub>50</sub> = 0.34 mmol·L<sup>-1</sup>) while being greater than those of lidocaine and cocaine (IC<sub>50</sub> = 0.74 and 0.80 mmol·L<sup>-1</sup> respectively).

### *Clinical significance of the effect of dexmedetomidine on nerve CAPs*

In humans, dexmedetomidine produces sedation and analgesia, and decreases heart rate, cardiac output and memory; each of these dexmedetomi-

dine actions depends on its plasma concentrations in a different manner (Ebert *et al.*, 2000). In patients, sedation is rapidly produced by 0.2 to 0.7  $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$  i.v. (Bhana *et al.*, 2000). In veterinary use, when injected intramuscularly in cats, 40  $\mu\text{g}\cdot\text{kg}^{-1}$  is a usual dose for sedation/analgesia (Slingsby and Taylor, 2008). Our study demonstrated a detail of the local anaesthetic effect of dexmedetomidine. The concentrations of dexmedetomidine, enough to inhibit nerve conduction, are mostly over 1000-fold higher than those used for dexmedetomidine as  $\alpha_2$  adrenoceptor agonist, as clinical doses of dexmedetomidine give plasma levels below 0.05  $\mu\text{mol}\cdot\text{L}^{-1}$  (see Ebert *et al.*, 2000). Thus, any effects of dexmedetomidine on nerve conduction is quite separate from the most current use of dexmedetomidine for sedation or analgesia.

$\alpha_2$ -Adrenoceptor agonists including dexmedetomidine, combined with a local anaesthetic, have been used to extend the duration of peripheral nerve conduction block (Singelyn *et al.*, 1996; Madan *et al.*, 2001; Memiş *et al.*, 2004; Calasans-Maia *et al.*, 2005; Kanazi *et al.*, 2006). This action is suggested to be due to a local vasoconstriction leading to a delay of the absorption of the local anaesthetic and/or a direct inhibition of nerve conduction by  $\alpha_2$  agonists (Tschernko *et al.*, 1998). The latter mechanism may be supported by a nerve conduction inhibition by dexmedetomidine, as revealed in the present study.

In conclusion, dexmedetomidine produces a conduction block with a higher potency than that of the local anaesthetic lidocaine. This action is relevant when considering the topical application of dexmedetomidine on nerves, but is not related to dexmedetomidine use for sedation, anaesthesia or analgesia by systemic administration. Conduction block was observed after other  $\alpha_2$ -adrenoceptor agonists, oxymetazoline and clonidine, and also the  $\alpha_2$ -adrenoceptor antagonist atipamezole. A chemical structure related to the  $\alpha_2$  adrenoceptor drugs may play an important role in producing nerve conduction block. The molecular targets of the  $\alpha_2$  adrenoceptor drugs, in this context, remain to be determined.

### Conflicts of interest

None.

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