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Action of dexmedetomidine on rat locus coeruleus neurones: intracellular recording in vitro

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Abstract

The action of dexmedetomidine on rat locus coeruleus neurones was examined using intracellular recordings from the in vitro brain slice preparation. Concentrations of dexmedetomidine from 1 to 1000 nM were tested. At 30 nM, dexmedetomidine produced complete inhibition of firing of all neurones tested ($n = 21$); this was associated with a 13 mV hyperpolarization (range 2.2–29.7 mV, $n = 21$) and a 27% reduction in input resistance (range 11.1–46.2%, $n = 17$). The dexmedetomidine responses reached a plateau phase between 100 and 1000 nM. Based on single-cell recordings, the hyperpolarizing potency of dexmedetomidine was found to be 6 times greater than that of clonidine ($n = 10$). The reversal potential for the dexmedetomidine-induced hyperpolarization was -106.9 ± 1.7 mV ($n = 9$), a value similar to the K^+ equilibrium potential; hyperpolarization was blocked by both CsCl and $BaCl_2$. The effect of dexmedetomidine was antagonized by yohimbine, with a dissociation equilibrium constant of 30 nM. In contrast, prazosin, the α_1-, α_{2B}- and α_{2C}-adrenoceptor subtype-preferring ligand, did not inhibit the dexmedetomidine effect. Our results also show that low concentrations of oxymetazoline (10–300 nM), an α_{2A}-adrenoceptor subtype-selective drug, cause profound inhibition of neuronal activity in the locus coeruleus. These data therefore suggest that dexmedetomidine binds to α_{2A}-adrenoceptors on the cell membrane of neurones of the locus coeruleus and that this leads to opening of the inwardly rectifying K^+ channels, resulting in the observed hyperpolarization of the membrane.

Keywords: Dexmedetomidine; Locus ceruleus; Brain slice; Intracellular recording; α_{2A}-Adrenoceptor

1. Introduction

There has recently been substantial interest in the use of α_2-adrenoceptor agonists in clinical anaesthesiology (for reviews see Hayashi and Maze, 1993; Pertovaara, 1993). Numerous studies have demonstrated that clonidine, an α_2-adrenoceptor agonist with an α_2-adrenoceptor/α_1-adrenoceptor binding ratio of 200:1, promotes analgesia and anaesthesia in humans (see Hayashi and Maze, 1993 for references). However, clonidine is not the most suitable α_2-adrenoceptor agonist for use in clinical studies because of its α_1-adrenoceptor agonist properties, which limit its anaesthetic action (e.g., see Bloor and Flacke, 1982). This ceiling phenomenon observed with clonidine has led to a search for more potent and specific α_2-adrenoceptor agonists. Dexmedetomidine, a new potent and highly selective α_2-adrenoceptor agonist, has analgesic and sedative properties in vivo (for reviews see Peden and Prys-Roberts, 1992; Pertovaara, 1993). Compared with clonidine, it has 7-fold greater selectivity for α_2- versus α_1-adrenoceptors and acts as a full agonist in most pharmacological test models (Virtanen et al., 1988). Data from animal studies have demonstrated that dexmedetomidine can be used as the sole anaesthetic (Vickery et al., 1988; Maze et al., 1988). In humans, the therapeutic value of dexmedetomidine as an anaesthetic agent has been explored in phase II (in the USA) and phase III (in Europe) clinical trials (Hayashi and Maze, 1993).

Although dexmedetomidine has great potential for wide use in human anaesthesia, its cellular mechanism of action on the central nervous system has not yet been entirely elucidated. We have carried out a detailed assessment of the actions of dexmedetomidine

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on neurones of the locus coeruleus using intracellular recording analysis techniques. The locus coeruleus is a compact group of norepinephrine-containing cell bodies close to the floor of the fourth ventricle at the upper border of the pons and projections from this small pontine nucleus give rise to more than half the noradrenergic neurones in the brain (Amaral and Sinnamon, 1977). The locus coeruleus brain slice was used in the present investigation for three reasons. Firstly, since this pontine nucleus possesses a high density of α_2-adrenoceptor binding sites, it provides an excellent site for studying the actions of α_2-adrenoceptor agonists (Aghajanian and VanderMaelen, 1982; Williams et al., 1985). Secondly, the locus coeruleus is the primary site of origin of descending noradrenergic analgesic fibers. Involvement of the locus coeruleus in α_2-adrenoceptor-mediated antinociception has been suggested (for reviews see Proudfit, 1988; Lipp, 1991; Jones, 1991). Thirdly, several recent studies have demonstrated that the locus coeruleus is a major neuroanatomical region at which dexmedetomidine exerts its sedative-anaesthetic actions (Correa-Sales et al., 1992a,b; Pertovaara et al., 1994). Furthermore, in this study the receptor subtype involved in the dexmedetomidine-induced effects was characterized by the use of subtype-selective ligands, oxymetazoline (selective for the α_{2A}-adrenoceptor) and prazosin (selective for α_1-, α_{2B}- and α_{2C}-adrenoceptors).

2. Materials and methods

2.1. Preparation and maintenance of slices of locus coeruleus

The methods used in preparing and maintaining slices of rat locus coeruleus were similar to those previously described (Shefner and Chiu, 1986; Chiu et al., 1990). Male Sprague-Dawley rats (120–200 g) were anaesthetized with ether and their brains were rapidly removed. A block of tissue containing the pons was excised and attached to a small Plexiglass stage with cyanoacrylate glue; an agar block, next to the tissue, served to support it during sectioning. The tissue was then submerged in oxygenated artificial cerebrospinal fluid (artificial CSF), maintained at 3–5°C, in the well of a Lancer 1000 vibratome, and coronal slices of the pons (300–350 μm thick) were prepared. A slice containing a cross-section through the caudal end of the locus coeruleus was mounted in the recording chamber and allowed to equilibrate for 1 h. The recording chamber consisted of a Plexiglass bath (0.3 ml) with a nylon net stretched tightly across it. The slice was placed on a square of lens paper on top of the net and an electron microscopy grid, held down by platinum wires, was placed over the locus coeruleus to prevent the slice from floating. The slice was completely submerged in artificial CSF with the following composition (mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 26.2, $MgCl_2$ 1.3, $CaCl_2$ 2.4, glucose 11.1 (saturated with 95% O_2-5% CO_2, pH 7.4). The temperature of the artificial CSF was 33–34°C and the flow rate 2.3 ml min^{-1}.

2.2. Intracellular recording

The slice was viewed from above using a dissection microscope. In the trans-illuminated slice, the locus coeruleus is seen as a translucent area lying on the lateral aspect of the periventricular gray, below the fourth ventricle. Recording microelectrodes, filled with KCl (2 M) and with a d.c. tip resistance of 40–70 MΩ, were used. They were inserted into the locus coeruleus under visual control. Intracellular potentials were recorded using an amplifier with an active bridge circuit, permitting the injection of current through the recording electrode (WPI M707). Current and voltage traces were displayed on a storage oscilloscope (Textronix 5113) and a rectilinear pen recorder (Gould 2400). Input resistance was measured by passing pulses of hyperpolarizing current at various amplitudes; the resulting alterations of voltage were measured and voltage-current (V/I) curves were constructed. Current pulses were of sufficient duration (250–300 ms) to fully charge the membrane capacitance and to reach steady-state voltage deflection.

2.3. Perfusion of solutions and drugs

A valve system was used to switch the solutions superfusing the preparation from control artificial CSF to artificial CSF containing drugs. The period required for test solutions to reach the chamber was known and was in the range of 25–35 s. Drugs were administered for a long enough period to reach a steady-state response and the effects were noted 5 min or longer after the beginning of drug infusion. The following drugs were used: dexmedetomidine hydrochloride (Farmos), clonidine hydrochloride, oxymetazoline hydrochloride, yohimbine hydrochloride, prazosin hydrochloride, $BaCl_2$ and CsCl (Sigma). Numerical data are expressed as the means ± standard error of the mean (S.E.M.).

3. Results

The present results are based on recordings made from a total of 142 locus coeruleus neurones, with stable intracellular impalements. The cells had resting membrane potentials of −50 to −69.7 mV (−59.5 ± 0.3 mV, $n = 142$) and an apparent input resistance of 100–375 MΩ (165 ± 5 MΩ, $n = 132$). They fired spon-

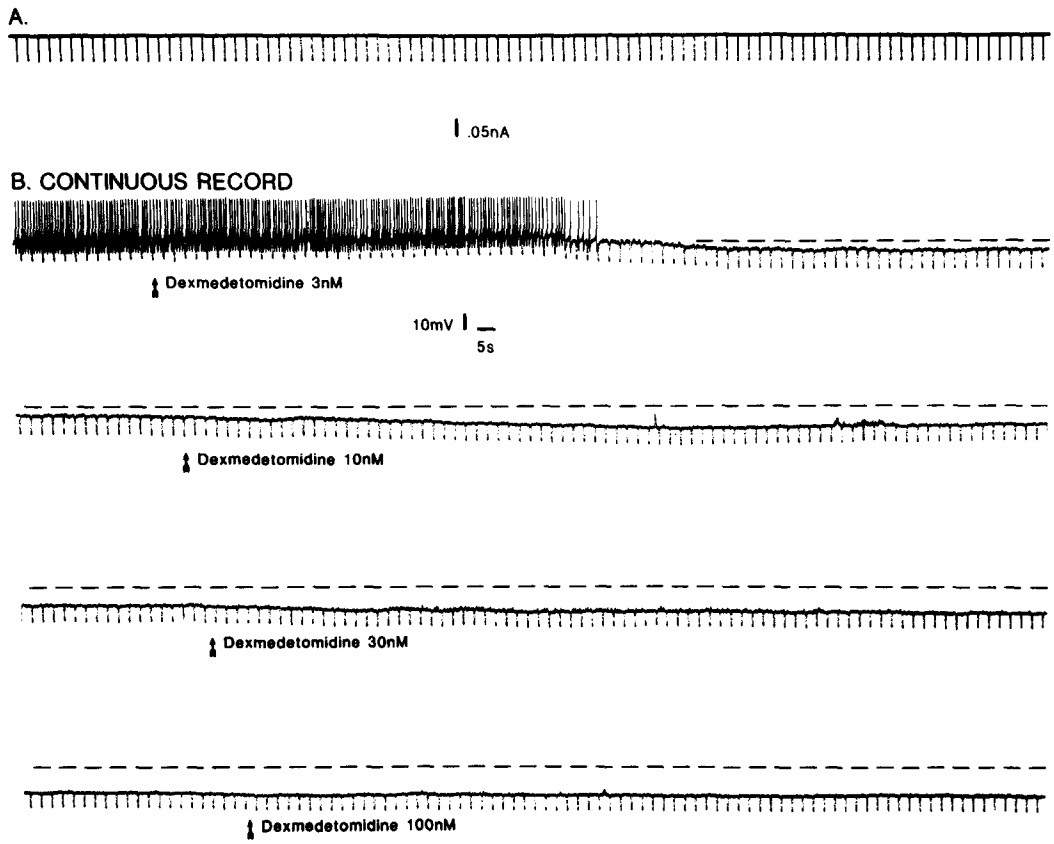

Fig. 1. The dose-dependent effect of the cumulative application of dexmedetomidine (3, 10, 30 and 100 nM) on the firing rate, membrane potential and input resistance of a typical locus coeruleus neurone. The upper panel shows injected currents and the lower four panels show a continuous record of a voltage trace. Broken horizontal lines indicate −55 mV.

taneously from 0.3 to 3.6 Hz (2.1 ± 0.1 Hz, $n = 142$), with a regular interspike interval characteristic of neurones of the locus coeruleus in the slice preparation (Williams et al., 1984).

3.1. Dexmedetomidine responses

The effect of dexmedetomidine was only partially reversible on washing the tissue with drug-free solution for periods up to 2 h. Dexmedetomidine was therefore applied cumulatively to each neurone and the data from many cells were pooled to construct the cumulative concentration-response curve. Fig. 1 shows the effect of four incremental concentrations of dexmedetomidine on a typical neurone in the locus coeruleus. While neurones varied in their sensitivity to dexmedetomidine, the inhibition of firing rate, hyperpolarization of the membrane potential and reduction in input resistance were concentration-dependent (Fig. 2). The most sensitive of these effects, which showed a remarkable change even at low dexmedetomidine concentrations, was the decrease in firing rate. Dexmedetomidine, 1 and 3 nM respectively, decreased the neuronal firing rate by 23% ($n = 25$) and 67% ($n =$ 21,including eight cells whose firing was completely suppressed); however, little hyperpolarization and only a small reduction of input resistance of the neurones were seen. Higher concentrations of dexmedetomidine (10–100 nM) resulted not only in a greater inhibition of firing but also in the hyperpolarization of the membrane and a decreased input resistance. Considerable variation was seen in the extent of hyperpolarization and reduction of input resistance induced by the same

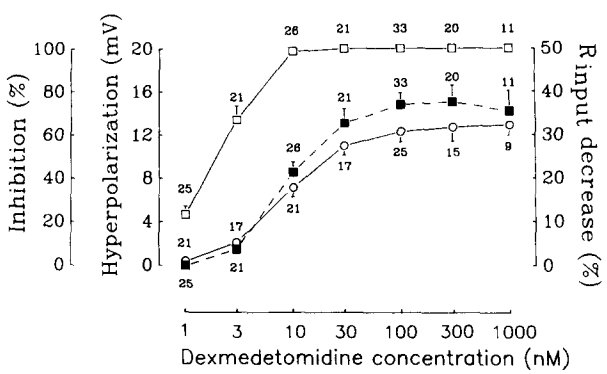

Fig. 2. Dose-dependent effects of dexmedetomidine on the firing rate (□), membrane potential (■) and input resistance (○) of neurones of the locus coeruleus. Means ± S.E.M. are shown for the number of neurones indicated.

concentration of dexmedetomidine in different neurones. For instance, the amplitude of hyperpolarization and reduction of input resistance in response to 10 nM dexmedetomidine ranged from 0 to 20.9 mV (8.5 ± 1.0 mV, $n = 26$) and from 0 to 31% (17.8 ± 2.2%, $n = 21$), respectively. 30 nM dexmedetomidine produced a complete inhibition of firing of all neurones tested ($n = 21$); this was associated with a 13 mV hyperpolarization (range 2.2–29.7 mV, $n = 21$) and a 27% reduction in input resistance (range 11.1–46.2%, $n = 17$). The dexmedetomidine responses reached a plateau phase between 100 and 1000 nM.

3.2. Comparison of potencies of dexmedetomidine, clonidine and oxymetazoline

Dexmedetomidine (3–300 nM), clonidine (10–300 nM) and oxymetazoline (10–300 nM) produced dose-related hyperpolarization in all locus coeruleus neurones tested; the data were used to construct dose-response curves. However, it was generally impracticable to construct full concentration-hyperpolarization sigmoidal curves for any two of these three drugs during the course of recording from a single cell. The rank order of potency of dexmedetomidine and clonidine was, therefore, determined by comparing the equi-effective concentrations that produced a 7 mV hyperpolarization (the half-maximal response caused by 100 nM dexmedetomidine; see Fig. 2). Fig. 3 shows the results from one of ten cells, showing that dexmedetomidine is 5.4 times more potent than clonidine to hyperpolarize locus coeruleus neurones (measured at a response level of 7 mV). For the ten neurones examined, a 2- to 10-fold lower concentration of dexmedetomidine was needed to produce an equivalent hyperpolarization, i.e. the equi-effective concentration of clonidine was 6.1 ± 1.1 times (range 2–10 times, $n = 10$) greater than that of dexmedetomidine. In another set

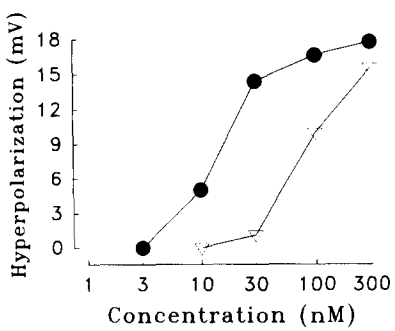

Fig. 3. The dose-response curves for dexmedetomidine (•) and clonidine (▽) for the hyperpolarization of resting potential in the locus coeruleus. The concentration required to produce a 7 mV hyperpolarization of the membrane potential was calculated as 12.6 and 67.6 nM for dexmedetomidine and clonidine, respectively. In this cell, dexmedetomidine was 5.4 times more potent than clonidine.

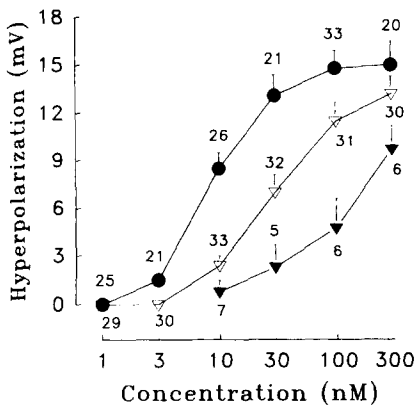

Fig. 4. The cumulative dose-response curves for dexmedetomidine (•), clonidine (▽) and oxymetazoline (▼) for the hyperpolarization of resting potential in a population of locus coeruleus neurones. The concentration required to produce a 7 mV hyperpolarization of the membrane potential was calculated as 7.3, 31.4 and 162.2 nM for dexmedetomidine, clonidine and oxymetazoline, respectively; the hyperpolarizing potency of dexmedetomidine was therefore 4.3 and 22.2 times more potent than that of clonidine and oxymetazoline.

of experiments, cumulative concentration-response curves for dexmedetomidine, clonidine and oxymetazoline for the membrane potential hyperpolarization of a population of locus coeruleus neurones were constructed (Fig. 4). These experiments showed that the hyperpolarizing potency of dexmedetomidine was 4.3 and 22.2 times greater than that of clonidine and oxymetazoline, respectively (measured at 7 mV response level; see Fig. 4). The equi-effective concentration ratio for clonidine over dexmedetomidine determined by the two methods showed good agreement.

3.3. Dexmedetomidine reversal potential

The fact that the dexmedetomidine reversal potential is extremely negative made it difficult to reverse dexmedetomidine responses by hyperpolarizing the membrane with d.c. current injection. Instead, the reversal potential for dexmedetomidine-induced hyperpolarization was calculated from the intersection of the control current-voltage curve and the curve obtained in the presence of dexmedetomidine. The relationship between injected current pulses and steady-state membrane potentials is shown in Fig. 5. The current-voltage relationship of the locus coeruleus neurones revealed an anomalous rectification that has been described previously (Andrade and Aghajanian, 1984; Osmanovic and Shefner, 1987; Williams et al., 1988). After dexmedetomidine administration, the slope of the current-voltage curve was reduced throughout the voltage range examined; the decreased input resistance elicited by dexmedetomidine did not therefore result simply from anomalous rectification by the cell membrane. The reversal potential of dexmedetomidine-induced

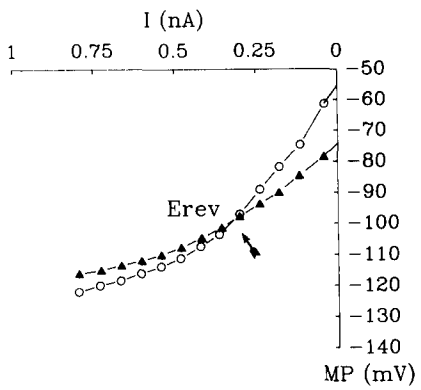

Fig. 5. Effect of dexmedetomidine on the current-voltage relationship of a neurone in the locus coeruleus. Open circles indicate data obtained in control artificial CSF and filled triangles represent data collected in artificial CSF containing 100 nM dexmedetomidine. The arrow indicates the reversal potential, which was −100 mV in this neurone.

hyperpolarization in each of ten neurones was deduced from the intersection of the two current-voltage curves. The values ranged from −100 to −116 mV, the average being −107 ± 2 mV ($n = 10$).

3.4. Effect of $BaCl_2$ and CsCl on blocking hyperpolarization caused by dexmedetomidine

The blocking effects of Ba^{2+} (0.3 mM) and Cs^+ (3 mM) on dexmedetomidine-induced hyperpolarization were tested on eight and ten neurones of the locus coeruleus, respectively. Ba^{2+} depolarized the membrane and increased the amplitude and duration of the action potential. Cs^+ also increased the peak amplitude, duration and afterhyperpolarization of the neuronal action potential. After superfusion with CsCl (3 mM) for 25 min or $BaCl_2$ (0.3 mM) for 15 min, the same neurone was challenged with dexmedetomidine (100 nM) in the presence of Cs^+ or Ba^{2+}. In the Cs^+-treated neurones, Cs^+ reduced, but did not eliminate, the dexmedetomidine-induced hyperpolarization; on average, a reduction of 50.6 ± 3.5% was seen. Ba^{2+}, on the other hand, completely blocked dexmedetomidine-induced hyperpolarization, but a partial suppression of the baseline firing rate was still observed (79.0 ± 5.7%, $n = 8$, including two cells in which firing was completely inhibited).

3.5. Interaction of dexmedetomidine with yohimbine and prazosin

To study the effects of yohimbine, a single concentration of yohimbine (300 or 1000 nM) was first applied before the perfusate was changed to the same fixed concentration of yohimbine plus dexmedetomidine at various concentrations (10–1000 nM). Because the dexmedetomidine-induced hyperpolarization reverses so slowly after washing, it was again necessary to construct cumulative concentration-response curves for dexmedetomidine in the presence or absence of yohimbine and to use pooled data from many experiments

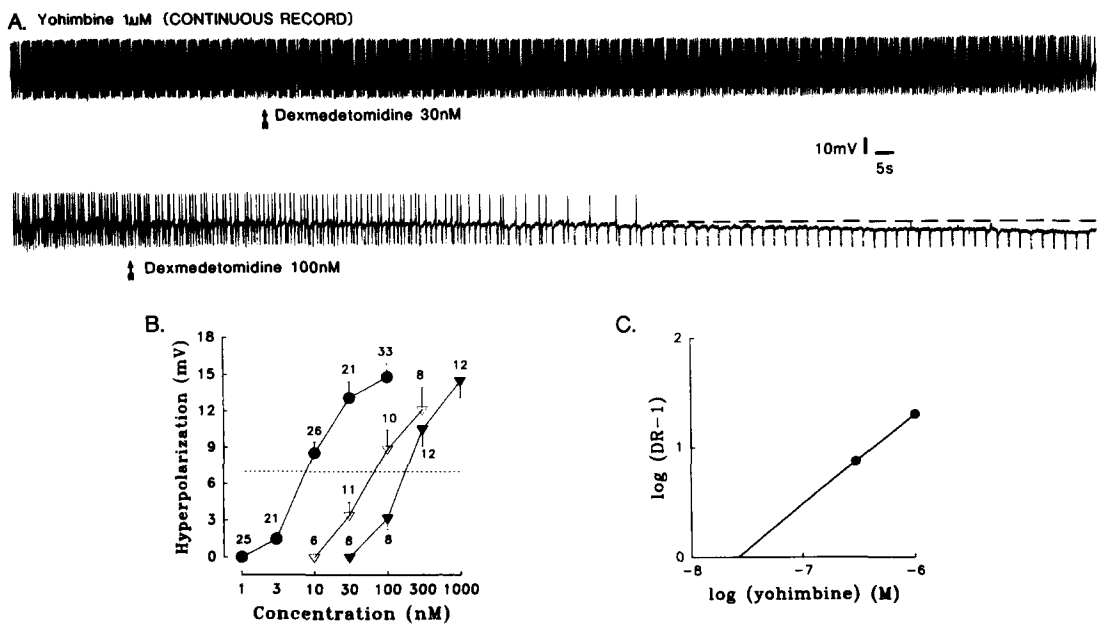

Fig. 6. Antagonism by yohimbine of dexmedetomidine-induced hyperpolarization. (A) Shows the effect of two different concentrations of dexmedetomidine (30 and 100 nM) on the locus coeruleus neurone in the presence of yohimbine (1 μM). (B) Cumulative dose-response curves for dexmedetomidine in the presence of different concentrations of yohimbine: control (●); yohimbine 300 nM (▽) and 1 μM (▼). (C) Schild plot of data shown in (B), at a response level of 7 mV. The dissociation equilibrium constant was 30.4 nM for the yohimbine antagonism of dexmedetomidine-induced hyperpolarizations.

(Fig. 6B). Yohimbine produced a parallel, dose-related shift to the right of the dexmedetomidine concentration-response curve. To determine the dissociation equilibrium constants (K_d) for yohimbine, we calculated the dose-ratios by dividing the concentration of dexmedetomidine required to produce a given hyperpolarization in the presence of yohimbine by the concentration of dexmedetomidine required to produce the same hyperpolarization in the absence of yohimbine. These dose-ratios were then used to construct the Schild plots (Fig. 6C), as described by Williams and North (1984). The experiments gave a K_d value of 30.4 nM for the antagonism of dexmedetomidine-induced hyperpolarizations by yohimbine. Prazosin (1 μM), on the other hand, did not antagonize the hyperpolarization response of locus coeruleus to various concentrations of dexmedetomidine (300 nM,$n = 8$; 100 nM, $n = 9$; 30 nM, $n = 9$; 10 nM,$n = 9$).

4. Discussion

Previous studies, with animal experiments and with brain slice preparations, have shown that neurones in the locus coeruleus are inhibited by α_2-adrenoceptor agonists (Cedarbaum and Aghajanian, 1977; Aghajanian and VanderMaelen, 1982; Egan et al., 1983; Williams et al., 1985). The present data provide evidence that dexmedetomidine, a highly selective α_2-adrenoceptor agonist, is able to inhibit the spontaneous firing rate, cause hyperpolarization of the membrane potential and reduce the input resistance of neurones of the locus coeruleus. Using extracellular single neurone recording and voltammetric measurement, it has been demonstrated that dexmedetomidine decreases both noradrenaline release and neuronal firing rate in a concentration-dependent manner in isolated brain slices of the rat locus coeruleus (Jorm and Stamford, 1993). The present results for spontaneous neuronal activity are compatible with the extracellular findings. We also found that the depressant effects of dexmedetomidine (an imidazole, like clonidine) on neurones of the locus coeruleus were more potent than those of the two clinically used imidazoline derivatives, clonidine and oxymetazoline. Comparison of the ability of dexmedetomidine, clonidine and oxymetazoline to produce hyperpolarization in the locus coeruleus showed that dexmedetomidine was 6 and 22 times as potent as clonidine and oxymetazoline, respectively. This finding is in line with results of previous receptor binding studies, which indicate that dexmedetomidine has an affinity for the α_2-adrenoceptor which is 7 times greater than that of clonidine (see Introduction).

Regarding the mechanism of the hypnotic-anaesthetic action of dexmedetomidine, Doze et al. (1990) have demonstrated in rats that it can be attenuated by pretreatment with either pertussis toxin, a specific inactivator of inhibitory G-proteins, or 4-aminopyridine, a K^+ channel blocker. The present investigation has further characterized the receptor-effector mechanism involved in the action of dexmedetomidine. Dexmedetomidine-induced hyperpolarization of neurones of the locus coeruleus is apparently due to the opening of K^+ channels which display anomalous rectification; the reasoning for this has four parts: (1) the reversal potential of the dexmedetomidine effect is in agreement with the calculated K^+ equilibrium potential (Williams et al., 1988); (2) the current-voltage relationship shows an inward rectification both in the absence and presence of dexmedetomidine; (3) dexmedetomidine reduces the slope of the current-voltage curve throughout the voltage range examined; and (4) Ba^{2+} and Cs^+ suppress the dexmedetomidine-induced hyperpolarization. Our data are consistent with those from previous studies which indicated that α_2-adrenoceptor agonists open inwardly rectifying K^+ channels (Egan et al., 1983; Williams et al., 1985; Andrade and Aghajanian, 1985; Aghajanian and Wang, 1987). The functional significance of this hyperpolarization would be to increase the threshold of impulse initiation and thus to decrease the probability of a cell discharging in response to a given excitatory synaptic input. The mechanisms by which the locus coeruleus is involved in the mediation of sedation and analgesia are complicated, as there appears to be interaction between the supraspinal systems. A possible mechanism has been proposed, based on previous studies on locus coeruleus-mediated sedation (Ruffolo et al., 1993) or supraspinal analgesia (Chiu et al., 1993).

The present observation that yohimbine is able to counteract the inhibitory effect of dexmedetomidine on neurones of the locus coeruleus with a K_d value of 30 nM is consistent with a previous report which demonstrated that the K_d values for the antagonism of clonidine-induced hyperpolarization by various α_2-adrenoceptor antagonists ranged from 9 to 49 nM (Williams et al., 1985). As different α_2-adrenoceptor subtypes may have distinct physiological functions in the brain, classifications of α_2-adrenoceptors have been based on either pharmacological (α_{2A}, α_{2B} or α_{2C}) or molecular biological (α_2-C10, α_2-C2 or α_2-C4) approaches (Bylund, 1988). However, recently it has proved possible to equate the receptor types in these two systems of classification with α_{2A}, α_{2B} and α_{2C} corresponding to α_2-C10, α_2-C2 and α_2-C4, respectively (Bylund, 1992; Bylund et al., 1992). Maze and Tranquilli (1991) have indicated that the anaesthetic effects of α_2-adrenoceptor agonists may be subtype-specific. It is therefore of interest to know which α_2-adrenoceptor subtype is involved in dexmedetomidine-induced sedative-anaesthetic actions. Using molecular biological tech-

niques, Regan et al. (1989) have shown that dexmedetomidine binds with a 7-fold greater affinity to the α_2-C4 (α_{2C}-) than to the α_2-C10 (α_{2A}-) adrenoceptors and they speculated that the α_2-C4 adrenoceptor is probably the receptor involved in mediating the anaesthetic action of α_2-adrenoceptor agonists (Regan et al., 1989; Maze and Regan, 1991). However, the present work suggests a major role for the α_{2A}-adrenoceptor in mediating the actions of dexmedetomidine. Firstly, low concentrations of oxymetazoline (10-300 nM), an α_{2A}-adrenoceptor subtype-selective drug (Bylund, 1988, 1992), elicit profound inhibition of neuronal activity in the locus coeruleus. Secondly, prazosin, which, in addition to its classic action as an α_1-adrenoceptor antagonist, has been shown to have a preferential affinity for α_{2B}- and α_{2C}-adrenoceptors (Bylund, 1988, 1992), does not antagonize the action of dexmedetomidine on the locus coeruleus. Therefore, as far as the α_2-adrenoceptor subtype is concerned, our electrophysiological data are in agreement with results of recent studies which have shown that α_2-adrenoceptors in the locus coeruleus are of the α_{2A} subtype. These studies employed immunohistochemical techniques (e.g., see Guyenet et al., 1994), autoradiographic techniques (e.g., see Ordway et al., 1993) or molecular biological techniques (e.g., see Nicholas et al., 1993). Recent studies with dexmedetomidine (Takano and Yaksh, 1993) or other α_2-adrenoceptor agonists (Millan et al., 1994) have also demonstrated a mediating role for the α_{2A}-adrenoceptor in antinociceptive or sedative mechanisms.

In conclusion, the present data indicate that the high potency of dexmedetomidine suggests a prospective therapeutic application for this selective α_2-adrenoceptor agonist. Additionally, the present and previously cited results strongly support the concept of a preferential involvement of the α_{2A}-adrenoceptor in sedative-anaesthetic mechanisms, although we do not definitely rule out the involvement of other α_2-adrenoceptor subtypes.

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