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Short communication

Synthesis and neuromuscular blocking activity of 16β-*N*-methylpiperazino steroidal derivatives

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Abstract

Steroidal quaternary ammonium compounds **12** and **13** with quaternised nitrogen at positions 3 and 16 of the steroidal nucleus in androstane series were synthesised and their neuromuscular blocking activities and ganglion blocking activities were studied using chick biventer and anaesthetised cat as the models. The bisquaternary compounds **12** and **13** have been found to be greater in potency than D-tubocurarine. Acetoxy derivative **13** has been found to be more potent than pipecuronium bromide taking D-tubocurarine as the standard compound indicating the need of acetoxy function at position 16.

Keywords: *N*-Methylpiperazine; Chick biventer; D-Tubocurarine; Non-depolarising; Bisquaternary; Neostigmine

1. Introduction

Neuromuscular blocking agents inhibit the nervous transmission at motor nerve endings in skeletal muscle resulting in muscle relaxation. They are used clinically as adjuncts to general anaesthesia to provide skeletal muscle relaxation for surgical procedures and are used to facilitate intubation procedures in orthopaedics for manipulation of fractured or dislocated bones [1,2], management of spasticity [3,4], control convulsions in tetanus and electroconvulsive therapy of psychiatric disorders [2]. The use of neuromuscular blockers in patients requiring mechanical ventilation as a part of intensive care [5] and management of muscle cramps [6] has been reviewed. These neuromuscular blocking agents act either by depolarisation of the motor end plate (e.g. suxamethonium) [7] or by competition with the chemical transmitter, acetylcholine at the neuromuscular junction (e.g. pancuronium bromide **1**) [8,9]. The discovery of D-tubocurarine **2** [10,11] and malouetine **3** [12] provided the key to the development of clinically useful depolarising and non-depolarising neuromuscular blocking agents. Pancuronium bromide **1**, chandonium iodide **4** and pipecuronium bromide **5** are the result of recent work in the various laboratories [13–15] (Fig. 1). The interonium distance has been reported [2,16,17]. It is widely held that optimal interonium distance between two quaternary heads for good neuromuscular blocking activity is 1.0–1.2 nm [16]. But, pipecuronium bromide **5**, a non-depolarising neuromuscular blocker does not fall within this parameter [18]. It is a long-acting competitive muscle relaxant with no significant cardiovascular side effects and histamine related effects [15,19–22]. We thus, thought it worthwhile to develop bisquaternary steroidal compounds with quaternised pyrrolidine at 3 position and quaternised *N*-methylpiperazino moiety at 16β position of the steroid nucleus, which we report here within this communication.

2. Chemistry

17-Oxo-5-androsten-3β-ol was brominated using cupric bromide in dry methanol to obtain 16α-bromo-17-oxo-5-androsten-3β-ol (**6**) [23]. The NMR spectrum exhibited a triplet centered at δ 4.46 (1H, 16β-*H*) and a multiplet at δ 5.30 (1H, 6-*H*) which indicated the C-5 olefin intact. The configuration at position 16 is in

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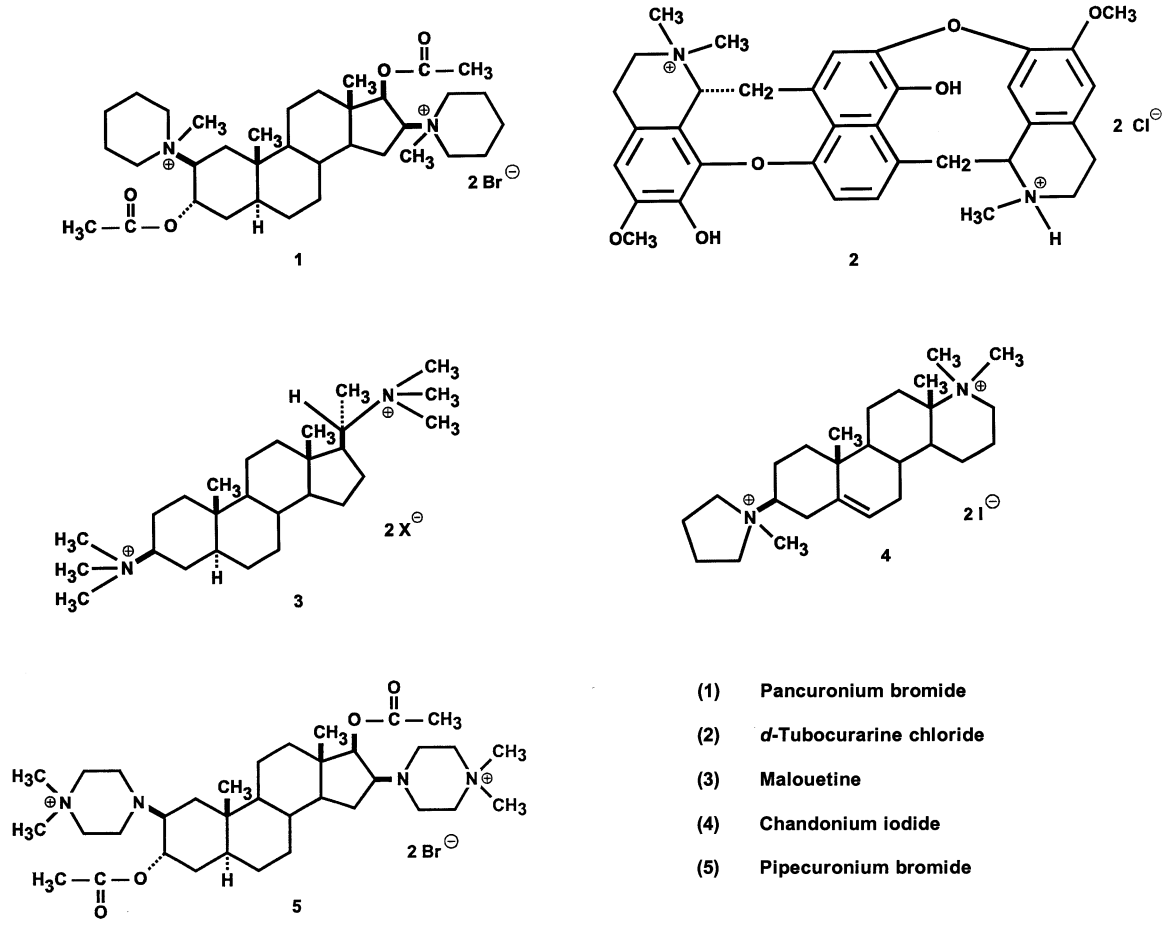

Fig. 1. Formulas of compounds **1**–**5**.

accordance with the earlier reports [23,24]. To fix the *N*-methylpiperazino functionality at position 16, the 16α-bromo derivative **6** was refluxed with *N*-methylpiperazine to give 16β-*N*-methylpiperazino derivative **7**. In the NMR spectrum a 3-proton singlet appeared at δ 2.28 ($>$N–CH_3) and a one proton multiplet centered at δ 3.08 (16α-*H*). Vibrational band at 1730 cm^{-1} indicated the 17-oxo function.

To prepare the bisquaternary compounds in the series 16β-*N*-methylpiperazino-17-one derivative **7** was subjected to Oppenauer oxidation using a cyclohexanone–toluene system to give the 4-en-3-one **8** (UV maximum at 238 nm). The infrared spectrum showed stretching at 1730 cm^{-1}. The 4-en-3-one derivative **8** was refluxed with freshly distilled pyrrolidine in methanol to yield the enamine **9** (UV maximum at 273.8 nm). The NMR spectrum exhibited signals at δ 4.73 (s, 1H, 4-C*H*) and 5.03 (m, 1H, 6-C*H*). Sodium borohydride reduction of the enamine **9** in methanol at room temperature yielded 16β-*N*-methylpiperazino-3β-pyrrolidino-5-androsten-17β-ol (**10**) [25]. The NMR spectrum showed the vinylic proton at δ 5.27. Acetylation of 3β-pyrrolidino derivative **10** with acetic anhyride in pyridine gave **11** (Fig. 2). The proton resonance signals appeared at δ 2.07 (s, 3H, –OCOCH_3), 2.23 (s, 3H, $>$N–CH_3), 3.0 (m, 1H, 16α-*H*) and 4.76 (d, 1H, $J = 9$ Hz, 17α-*H*). The bistertiary amine **10** was quaternised with methyl iodide in dry acetone at room temperature to yield the bisquaternary compound **12** (DPJ-494). The ^{1}H-NMR signals appeared at δ 2.95 (s, 3H, $>N$–CH$_3$ of piperazino moiety) and 3.16 (s, 6H, $>N$–CH$_3$ of piperazino moiety and pyrrolidino function). The quaternisation of the 17-acetoxy derivative **11** with methyl iodide in dichloromethane at room temperature yielded 16β-*N*-methylpiperazino-3β-pyrrolidino-5-androsten-17β-yl acetate dimethiodide **13** (DPJ-496) (Fig. 3). Its ^{1}H-NMR signals appeared at δ 2.08 (s, 3H, –OCOCH_3), 2.89 (s, 3H, $>N$–CH$_3$ of piperazino moiety) and 3.12 (s, 6H, $>N$–CH_3 of piperazino moiety and pyrrolidino function).

3. Neuromuscular blocking activity

The neuromuscular blocking activity of compounds **12** and **13** was investigated on chick biventer cervicis muscle preparations. The in vivo neuromuscular blocking actions and cardiovascular effects of the compounds

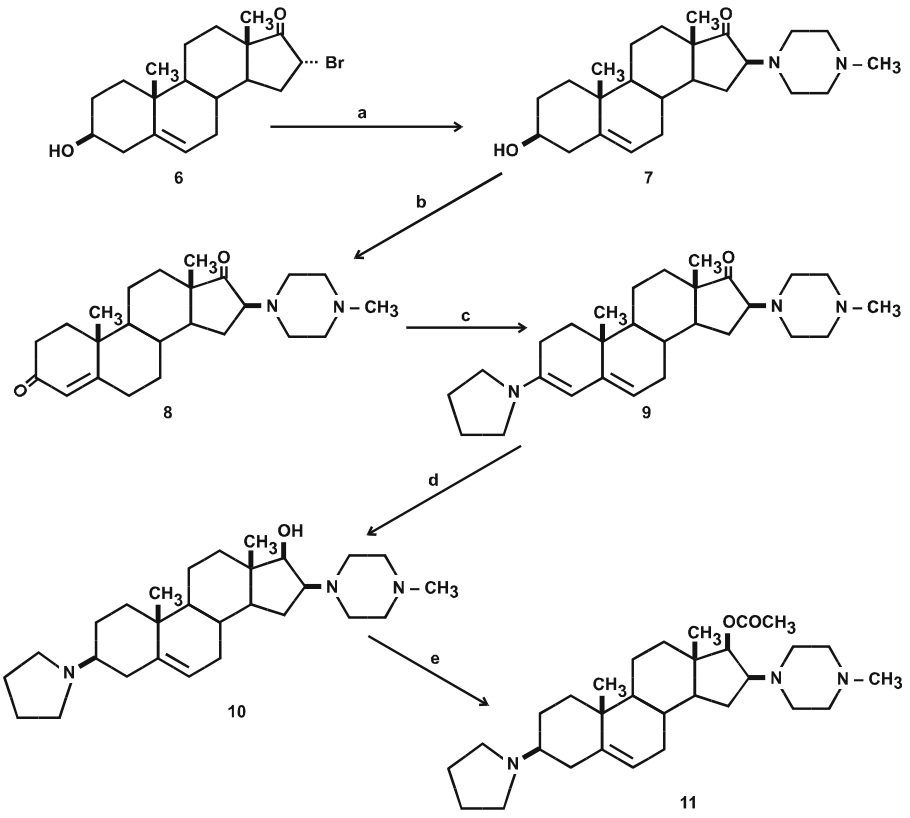

Fig. 2. Synthetic procedure of compounds **6**–**11**. Reagents and conditions: (a) *N*-methylpiperazine, reflux; (b) cyclohexanone–toluene–aluminium isopropoxide, reflux; (c) pyrrolidine–methanol, reflux; (d) sodium borohydride–methanol at room temperature; (e) acetic anhydride–pyridine, reflux.

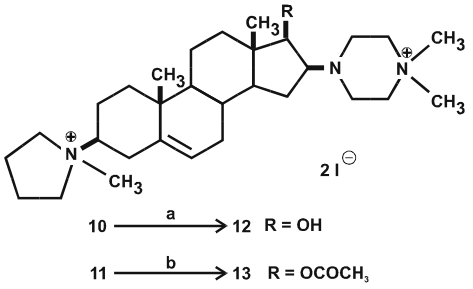

Fig. 3. Synthetic procedure of compounds **12** and **13**. Reagents and conditions: (a) methyl iodide–acetone at room temperature; (b) methyl iodide–dichloromethane at room temperature.

were examined in a single anaesthetised cat experiment. The competitive nature of the neuromuscular blocking effects of the compounds was determined by examining the ability of neostigmine to restore indirectly elicited twitch force.

4. Results

Compounds **12** and **13** were tested for their ability to produce an inhibition of indirectly elicited contractions of the chick biventer cervicis skeletal muscle preparation. Both compounds reduced nerve-evoked contractions of the muscle without producing any change in the baseline tension of the muscle. This latter observation is indicative of a lack of agonist effect of either compound at the post-junctional nicotinic acetylcholine receptors. To determine the neuromuscular blocking potency of the compounds, the concentration reducing the indirectly elicited twitch force by 70–95% was determined. Data obtained for the two compounds is shown in Table 1 along with data for D-tubocurarine as a reference standard. The competitive nature of the neuromuscular blocking effects of the compounds was determined by examining the ability of the anticholinesterase agent neostigmine (0.5 M) to restore indirectly elicited twitch force in the presence of the 70–95% blocking concentration of the compound (Table 1). The neuromuscular block produced by compound **13** was largely reversed by neostigmine, suggesting that the neuromuscular blocking action was predominantly due to a competitive antagonism of the post-junctional nicotinic acetylcholine receptors. For compound **12**, neostigmine produced an appreciable, but less complete, restoration of twitch force. Thus is possible that the lower potency of this compound might be associated with a more complex profile of neuromuscular blocking activity.

Table 1
Neuromuscular blocking action of compounds **12** and **13** in the chick biventer cervicis skeletal preparation

Compound	Number of muscles	Concentration (μM)	Maximum inhibition (%)	Relative potency [a]	Reversal neostigmine (%) [b]
Tubocurarine	6	1.00±0.13	–	1	–
12 (DJP-494)	10	0.50±0.07	83.4±3.1	2	46.6±3.5
13 (DJP-496)	7	0.02±0.03	88.4±1.9	50	68.2±5.1

[a] Indicates the neuromuscular blocking potency relative to tubocurarine (1 μM taken as a value of 1).
[b] Indicates the extent to which twitch force is returned to the control value in the presence of 0.5 μM neostigmine (100% would indicate the twitch force was fully restored to the control.

Because of its high potency relative to D-tubocurarine and its apparent competitive neuromuscular blocking activity, compound **13** alone was selected for further study in the chick biventer cervicis skeletal muscle preparation. The ability of a range of concentrations of the compound to inhibit carbachol-induced contractions of the muscle was determined. Compound **13** (0.1–0.3 M) produced a concentration-dependent parallel rightward shift of the carbachol concentration–effect curve with no apparent change in the maximum response to carbachol-indicative of competitive antagonism. However, analysis of the data revealed a slope of the Arunlakshana and Schild plot significantly different from unity (1.46 ± 0.09, $n = 5$) suggesting that this compound does have some non-competitive neuromuscular blocking action at the neuromuscular junction.

The in vivo neuromuscular blocking actions and cardiovascular effects of compounds **12** and **13** were examined in a single anaesthetised cat experiment. The ability of the compounds to block twitch force in the soleus muscle was measured and is shown in (Table 2). Data in the table is compared to previously published data [26] for two clinically available muscle relaxants, vecuronium and rocuronium, derived from identically executed experiments. Despite their very clear difference in potency in the in vitro chick biventer skeletal muscle preparations, in the anaesthetised cat, compounds **12** and **13** had a similar potency, being around 2–3 times more potent than rocuronium and around one-fourth the potency of vecuronium (Table 2). However, the two compounds showed differences in their time course of action. Compound **13** was considerably slower in onset than compound **12** and had a very long duration, being twice that of vecuronium. Conversely, the time course of action of compound **12** was unusually short for a compound with a suspected mechanism of action involving inhibition of post-junctional nicotinic acetylcholine receptors, being faster in action than the rapid-onset clinical muscle relaxant rocuronium.

Compound **13** had no effect on responses of the nictitating membrane, transiently elevated resting blood pressure and heart rate on injection and reduced the bradycardia response to stimulation of the vagus nerve by around one-third (Fig. 4). Compound **12** produced a much more severe effect on the cardiovascular system, including a lasting tachycardia and profound inhibition of the vagally induced bradycardia. Also, at a concentration producing an 80–95% block of twitch force in the soleus muscle, compound **12** produced an unexplained facilitatory response in the tibialis muscle (Fig. 5).

5. Discussion

There was a 25-fold drop in potency in the compounds in the chick biventer cervicis muscle in changing from the 17-acetate compound **13** (DPJ-496) to the 17-hydroxy compound **12** (DPJ-494). However, in the cat the two compounds were equipotent. The reason for this difference in the two species is not immediately obvious. One clear difference between the cat experiments and the chick experiments is that in the former, a compound is injected into a dynamic situation and blood flow and a

Table 2
Neuromuscular blocking action of compounds **12** and **13** in the soleus muscle of the anasthetised cat

Compound	Dose to produce 80–95% twitch block (mg kg^{-1})	Onset [a] (min)	Duration [b] (min)	Recovery [c] (min)
Vecuronium	38	5.0	17.4	5.2
Rocuronium	311	2.9	13.7	5.2
12 (DJP-494)	125	1.6	8.4	3.0
13 (DJP-496)	120	3.9	38.8	16.9

Values for vecuronium and rocuronium taken from Ref. [26].
[a] Onset = time from injection to maximum block.
[b] Duration = time from injection to 90% recovery.
[c] Recovery = time for recovery from 25 to 75% of initial twitch height.

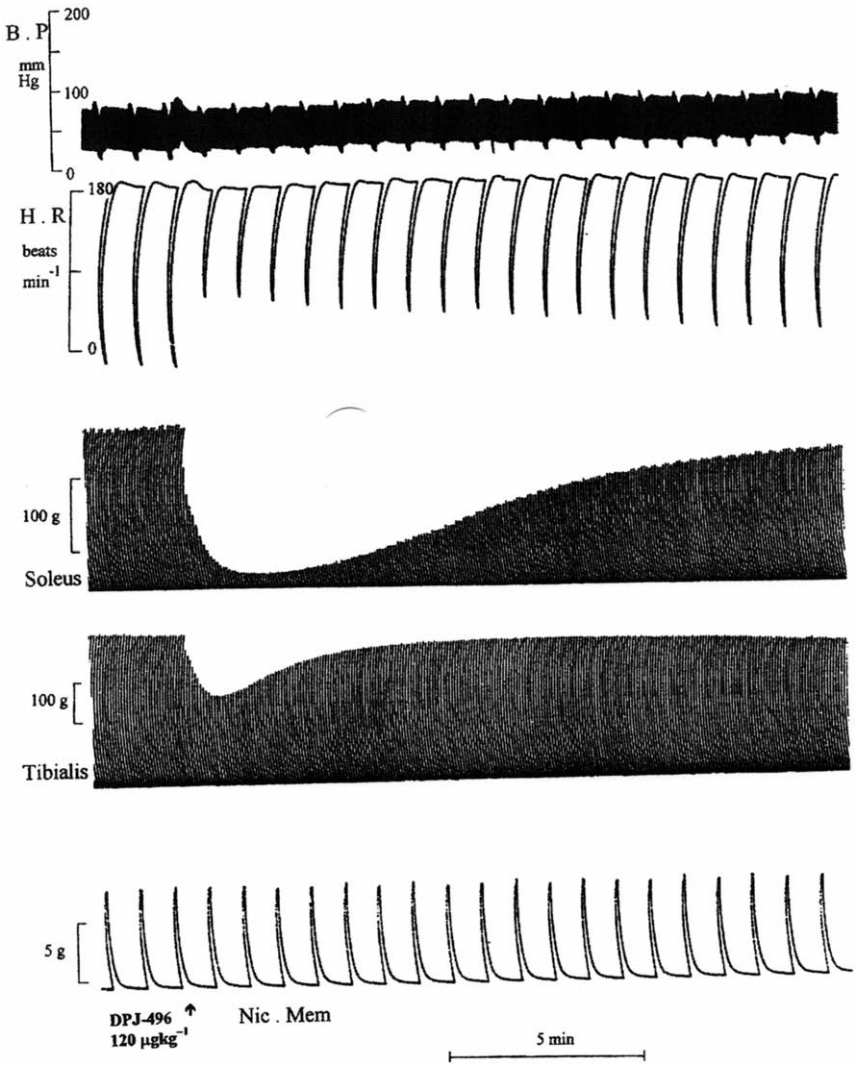

Fig. 4. Chloralose-anaesthetised cat. Effect of DPJ-496 (120 J μg kg^{-1}) on blood pressure (B.P), heart rate (H.R), response of the nictitating membrane (Nic.Mem) to preganglionic stimulation and responses of the soleus and tibialis anterior muscles to indirect stimulation at 0.1 Hz.

variety of pharmacokinetic factors will determine access to and removal from receptors and hence apparent potency. Clearly, the fact that two compounds with similar potencies in the cat have very different time courses of action would support the notion that the compounds have different pharmacokinetic profiles. In the chick, the compounds are added to an essentially static system and they depend upon diffusion through the preparation for access to the receptors. This is a relatively long process; e.g. 45 min can be required for maximum block to be achieved. Under these circumstances it is highly possible that non-receptor 'acceptor' sites may bind compounds and modify their apparent potency at the receptors. Another possibility is that the acetates tested in the cat, break down extremely rapidly on injection into the bloodstream, yielding their 17-hydroxy analogues as metabolites. For example, the 17-acetoxy grouping may be particularly susceptible to hydrolysis by plasma butyrylcholinesterase. If such a breakdown were to occur in plasma, it would not be expected to occur in the isolated chick muscle experiments where butyrylcholinesterase would not be a factor. Thus it is possible that the similar potencies of the two compounds tested in the cat may reflect their breakdown to the 17-hydroxy metabolites.

6. Experimental

6.1. Chemistry

The m.p.s reported are uncorrected. The ^{1}H-NMR spectra were recorded on AC-300F, 300 MHz, Varian EM-390, 90 MHz and EM-360, 60 MHz NMR instruments using tetramethylsilane (TMS) as the internal standard (chemical shifts in δ, ppm). The IR and UV spectra were recorded on Perkin–Elmer 882 and Lamda 15 spectrophotometer models, respectively. The purity

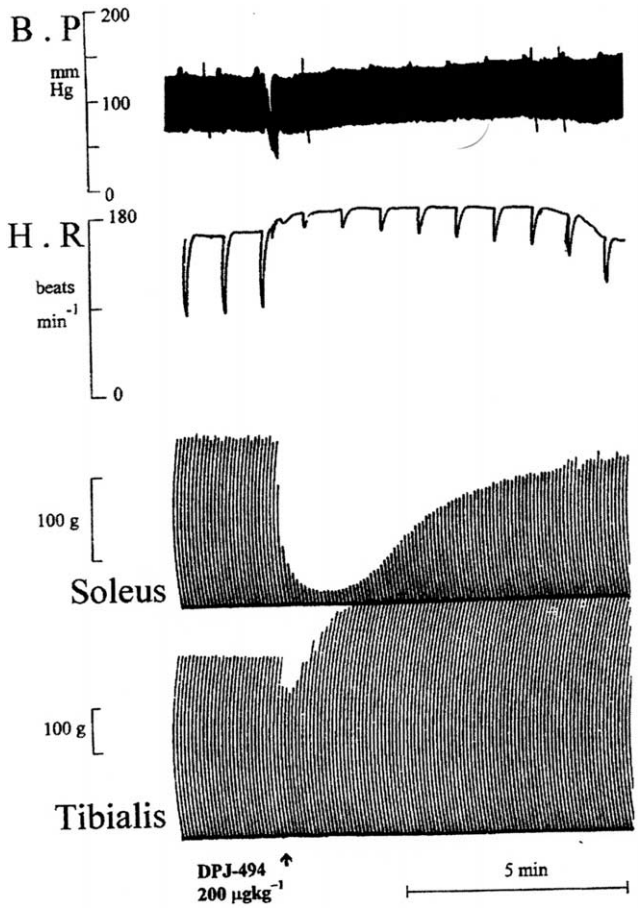

Fig. 5. Chloralose-anaesthetised cat. Effect of DPJ-494 (200 J μg kg^{-1}) on blood pressure (B.P) and heart rate (H.R) and responses of the soleus and tibialis anterior muscles to indirect stimulation at 0.1 Hz. Response of the nictitating membrane is not shown.

of the compounds was established by thin layer chromatography and by elemental analyses (C, H, N). Elemental analyses were carried out on a Perkin–Elmer-2400. Mass spectra were recorded on a V6-11-250J70S. Anhydrous Na_2SO_4 was used as a drying agent and iodine vapours as developing agent. Ultraviolet spectra were recorded in MeOH. IR spectra were obtained with KBr pellets (ν_{max} in cm^{-1}).

6.1.1. 16β-N-Methylpiperazino-17-oxo-5-androsten-3β-ol (7)

16α-Bromo-17-oxo-5-androsten-3β-ol (**6**) (1 g, 2.7 mmol) was refluxed with *N*-methylpiperazine (4.5 g, 44.9 mmol) for 1 h. Majority of the *N*-methylpiperazine was removed under reduced pressure and the product was precipitated with water. The solid material so obtained was filtered, washed with water and crystallised from C_3H_6O to afford **7** (0.65 g, 61.9%); m.p.: 160 °C; IR (C=O) 1730; ^{1}H-NMR ($CDCl_3$): δ 0.89 (s, 3H, 18-C*H*$_3$), 1.03 (s, 3H, 19-C*H*$_3$), 2.28 (s, 3H, >N–C*H*$_3$), 2.48–2.90 (m, 8H, protons of piperazino moiety), 3.08 (m, 1H, 16α-C*H*), 3.51 (m, 1H, 3α-C*H*) and 5.37 (d, 1H, 6-C*H*); MS: *m*/*z* 386 [M^+]. Anal. Calc. for $C_{24}H_{38}N_2O_2$: C, 74.57; H, 9.91; N, 7.25. Found: C, 74.31; H, 9.69; N, 6.88%.

6.1.2. 16β-N-Methylpiperazino-4-androstene-3,17-dione (8)

16β-*N*-methylpiperazino-17-oxo-5-androsten-3β-ol (**7**) (1.0 g, 2.59 mmol) was dissolved in a mixture of cyclohexanone (9.5 g, 96.8 mmol) and dry $C_6H_5CH_3$ (100 mL). Traces of moisture were removed by azeotroipic distillation of $C_6H_5CH_3$. The distillation was continued at a slow rate till the dropwise addition of a solution of aluminium isopropoxide (1.0 g, 4.9 mmol) in dry $C_6H_5CH_3$ (20 mL) was complete. The reaction mixture was refluxed for 4 h and allowed to stand at room temperature (r.t.) for 12 h. The slurry was filtered and filtrate was steam-distilled until the complete removal of organic solvents was effected. The residual aqueous suspension was allowed to stand at r.t. and extracted with $CHCl_3$ (4 × 25 mL). The combined $CHCl_3$ extract was washed with water, dried and the solvent removed to obtain a semisolid **8** which could not be crystallised and was used for further reaction without purification. UV_{max} (MeOH): 238 nm; IR: 1730 (C=O), 1660 (C=C–C=O); ^{1}H-NMR ($CDCI_3$): δ 0.90 (s, 3H, 18-C*H*$_3$), 1.0 (s, 3H, 19-C*H*$_3$), 2.30 (s, 3H, >*N*–C*H*$_3$), 2.47–2.88 (m, 8H, protons of piperazino moiety) and 5.80 (s, 1H, 4-C*H*).

6.1.3. 16β-N-Methylpiperazino-3-pyrrolidino-3,5-androstadien-17-one (9)

Freshly distilled pyrrolidine (4.3 g, 60.5 mmol) was added dropwise to a refluxing solution of 16β-*N*-methylpiperazino-4-androstene-3,17-dione (**8**) (1.0 g, 2.60 mmol) in MeOH (10 mL). Refluxing was further continued for 15 min and the solution was concentrated to induce crystallisation. The crystalline material was filtered, washed with MeOH and dried to give **9** (0.90 g, 78.95%); m.p.: 155–160 °C; UV_{max} (MeOH): 273.8 nm (log ε 3.36); IR: 1725 (C=O), 1590 (C=C stretch); ^{1}H-NMR ($CDCl_3$): δ 0.90 (s, 3H, 18-C*H*$_3$), 1.06 (s, 3H, 19-C*H*$_3$), 2.30 (s, 3H, >*N*–C*H*$_3$), 2.43–2.83 (m, 8H, protons of piperazino moiety), 2.90–3.23 (m, 5H, *N*-methylenes of pyrrolidino function, 16α-C*H*), 4.73 (s, 1H, 4-C*H*) and 5.03 (m, 1H, 6-C*H*). Anal. Calc. for $C_{28}H_{43}N_3O$: C, 76.84; H, 9.90; N, 9.60. Found: C, 76.41; H, 9.52; N, 9.41%.

6.1.4. 16β-N-Methylpiperazino-3β-pyrrolidino-5-androsten-17β-ol (10)

Sodium borohydride (2.0 g, 52.87 mmol) was added to a stirred suspension of 16β-*N*-methylpiperazino-3-pyrrolidino-3,5-androstadien-17-one (**9**) (2.0 g, 4.57 mmol) in MeOH (150 mL) in small quantities at r.t. Stirring was continued for 2 h, the reaction mixture was poured into ice-cold water (1000 mL), and the aqueous

suspension was extracted with $CHCl_3$ (4 × 100 mL). The combined $CHCl_3$ extract was washed with water, dried and solvent removed under reduced pressure to give a solid residue **10** (1.3 g, 64.4%); m.p.: 190 °C; IR: 3335 (O–H); ^{1}H-NMR ($CDCl_3$): δ 0.66 (s, 3H, 18-CH_3), 0.97 (s, 3H,19-CH_3), 2.23 (s, 3H, >N–CH_3), 2.47–2.83 (m, 8H, protons of piperazino moiety), 2.90–2.92 (m, 4H, *N*-methylenes of pyrrolidino function), 3.0–3.46 (m, 2H, 16α-C*H*, 17α-C*H*) and 5.27 (m, 1H, 6-C*H*); $[\alpha]_D^{22}$ −15.71° (*c* 0.042, $CHCl_3$); MS: *m/z* 442 [M^+]. Anal. Calc. for $C_{28}H_{47}N_3O$: C, 76.14; H, 10.73; N, 9.51. Found: C, 75.91; H, 10.56; N, 9.11%.

6.1.5. 16β-*N*-Methylpiperazino-3β-pyrrolidino-5-androsten-17β-yl acetate (*11*)

Compound **10** (0.5 g, 1.13 mmol) was heated in a mixture of Py (0.24 g, 3.1 mmol), and Ac_2O (0.54 g, 5.29 mmol) for 1 h on steam bath. The reaction mixture was poured into ice-cold water basified with NH_3 and the precipitated product was filtered, washed with water, dried and crystallised from C_3H_6O to give **11** (0.35 g, 63.9%); m.p.: 190 °C; IR: 1722 (–$OCOCH_3$), 1220 [C–(C=O)–O stretch]; ^{1}H-NMR ($CDCl_3$): δ 0.80 (s, 3H, 18-CH_3), 1.0 (s, 3H, 19-CH_3), 2.07 (s, 3H, –OCOCH_3), 2.23 (s, 3H, >N–CH_3), 2.33–2.83 (m, 8H, protons of piperazino moiety), 2.84–2.90 (m, 4H, *N*-methylenes of pyrrolidino function), 3.0 (m, 1H, 16α-C*H*), 4.76 (d, 1H, *J* = 9 Hz, 17α-C*H*) and 5.30 (m, 1H, 6-C*H*). Anal. Calc. for $C_{30}H_{49}N_3O_2$: C, 74.48; H, 10.21; N, 8.69. Found: C, 74.37; H, 10.08; N, 8.40%.

6.1.6. 16β-*N*-Methylpiperazino-3β-pyrrolidino-5-androsten-17β-ol dimethiodide (*12*) (*DPJ-494*)

Methyl iodide (9.08 g, 63.99 mmol) was added to a solution of 16β-*N*-methylpiperazino-3β-pyrrolidino-5-androsten-17β-ol (**10**) (2.0 g, 4.53 mmol) in dry C_3H_6O (20 mL) and the reaction mixture was kept at r.t. for 4 days. The reaction mixture was cooled and concentrated to induce crystallisation. The crystalline material was filtered, washed and dried to give **12** (2.2 g, 67.1%); m.p.: 290–295 °C; IR: 3360 (O–H), 1600 (C=C); ^{1}H-NMR (DMSO-d_6): δ 2.95 (s, 3H, >*N*–CH_3 of piperazino moiety) and 3.16 (s, 6H, >*N*–CH_3 of piperazino moiety, and >*N*–CH_3 of pyrrolidino function). Anal. Calc. for $C_{30}H_{53}I_2N_3O$: C, 49.66; H, 7.36; N, 5.79. Found: C, 49.50; H, 7.37; N, 5.36%.

6.1.7. 16β-*N*-Methylpiperazino-3β-pyrrolidino-5-androsten-17β-yl-acetate dimethiodide (*13*) (*DPJ-496*)

Quaternisation of 16β-*N*-methylpiperazino-3β-pyrrolidino-5-androsten-17β-yl acetate (**11**) (0.4 g, 0.83 mmol) in CH_2Cl_2 was done as above to afford **13** (0.40 g, 63%); m.p.: 250 °C; IR: 1715 (–$OCOCH_3$), 1225 [C–(C=O)–O Stretch]; ^{1}H-NMR (DMSO-d_6): δ 2.08 (s, 3H, 17β-OCOCH_3), 2.89 (s, 3H, >N–CH_3 of piperazino moiety) and 3.12 (s, 6H, >N–CH_3 of piperazino moiety and >N–CH_3 of pyrrolidino function). Anal. Calc. for $C_{32}H_{55}I_2N_3O_2$: C, 50.07; H, 7.22; N, 5.47. Found: C, 50.30; H, 7.12; N, 5.10%.

7. Pharmacological methods

7.1. Chick biventer cervicis muscle preparation

Biventer cervicis nerve–muscle preparations [27] were dissected from young chickens (3–10 days) and mounted in a 10 mL tissue bath maintained at 32 °C and containing Kreb's solution of the following composition (mmol l^{-1}): NaCl 118, KCl 5, $CaCl_2$ 2.5, $NaHCO_3$ 30, KH_2PO_4 1, $MgSO_4$ 1, glucose 11 and at pH 7.4 aspirated with 5% carbon dioxide in oxygen. The motor nerves were stimulated at a frequency of 0.1 Hz with rectangular pulses of 0.2 ms duration and a voltage greater than that required to produce maximal twitches. Tension responses were recorded by Grass FT03C force displacement transducers.

In some experiments dose–response curves were constructed by adding increasing concentrations of the agonist carbachol until maximal responses were obtained. Carbachol dose–response curves were constructed in the absence and the presence of three concentrations of the test compounds. Affinity constants (kD values) were calculated from plots of log (dose ratio-1) against molar concentration of antagonist [28]. Slope values from the plots were used to assess competitive antagonism or otherwise.

7.2. Anaesthetised cat

A cat was anaesthetised with a mixture of α-chloralose 80 mg kg^{-1} and pentobarbitone 5 mg kg^{-1} injected i.p. Lungs were ventilated with air at a rate of 26 b.p.m. at a tidal volume of ca. 13 mL kg^{-1}, adjusted to maintain arterial pH at 7.37–7.47.

The right hind limb was immobilised and the contractile responses of the tibialis anterior and soleus muscles to single shock stimulation of the sciatic nerve were recorded. The sciatic nerve is stimulated at a rate of 0.1 Hz using rectangular pulses of 0.2 ms duration and of length greater than that required to produce maximal twitch. This stimulation frequency was chosen to allow comparison of the results with test compounds with those for compounds reported previously in the literature. Contractions of the nictitating membrane were evoked in response to preganglionic stimulation of the cervical sympathetic nerve with trains (frequency 5 Hz, duration 10 s) of strength sufficient to produce maximal concentrations of nictitating membrane.

Arterial pressure was recorded from the carotid artery using a Statham PC45 pressure transducer. The arterial pressure pulse triggered a cardiachograph to display a

heart rate. Both vagus nerves were ligated and, at 100-s intervals, the right vagus nerve was stimulated at a frequency of 2–5 Hz and with pulses of 0.5 ms duration and strength greater than that required to produce a maximal reduction in heart rate. Contractile responses of muscle were recorded using Grass FTO3Cand FT10 displacement transducers. All responses were displayed on a Grass model 5 ink oscillograph.

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References

[1] J.B. Stenlake, in: G.P. Ellis, G.B. West (Eds.), Progress in Medicinal Chemistry, vol. 16, Elsevier, Amsterdam, 1979, pp. 257–286.
[2] A.R. Gennaro (Ed.), Remington: The Science and Practice of Pharmacy, vol. II, 20th ed., Lippincott Williams and Wilkins, A Wolters Kluwer Company, Philadelphia, 2001, pp. 1333–1343.
[3] R.R. Young, Neurology 44 (Suppl. 9) (1994) S12–S20.
[4] C. Koko, A.B. Ward, Br. J. Hosp. Med. 58 (1997) 400–405.
[5] J.M. Elliot, J.F. Bion, Acta Anaesthesiol. Scand. 39 (Suppl. 106) (1995) 70–82.
[6] S.R. McGee, Arch. Intern. Med. 150 (1990) 511–518.
[7] D.P. Cartwright, Br. J. Anaesth. 71 (1993) 918–919.
[8] J.M. Hunter, N. Engl. J. Med. 332 (1995) 1691–1699.
[9] T.M. Speight, G.S. Avery, Drugs 4 (1972) 163–226.
[10] H. King, J. Chem. Soc. (1935) 1381–1389.
[11] H.R. Griffith, G.E. Johnson, Anaesthesiology 3 (1942) 418–420.
[12] M.A. Quevauviller, M.F. Laine, Ann. Pharm. Fr. 18 (1960) 678–680.
[13] W.L.M. Baird, A.M. Reid, Br. J. Anaesth. 39 (1967) 775–780.
[14] W.R. Buckett, C.L. Hewett, D.S. Savage, J. Med. Chem. 16 (1973) 1116–1124.
[15] A. Barankay, Arzneim.-Forsch. Drug Res. 30 (1980) 386–389.
[16] W.C. Bowman, S.N. Webb, J. Pharm. Pharmacol. 24 (1972) 762–772.
[17] W.v. Phillipsborn, H. Schmid, P. Karrer, Helv. Chim. Acta 39 (1956) 913–922.
[18] W.C. Bowman, J. Szenohradszky, Gy. Marosi, I. Toth, Arzneim-Forsch. Drug Res. 30 (I) (1980) 389–393.
[19] S. Agoston, F.J. Richardson, Clin. Anaesthesiol. 3 (1985) 361–369.
[20] E. Kárpati, K. Biró, Arzneim-Forsch. Drug Res. 30 (1980) 346–354.
[21] R.N. Alyautdin, V.V. Buyanov, V.P. Fisenko, E.Ju. Lemina, V.K. Muratov, D.N. Somoilov, V.A. Shorr, Arzeim-Forsch. Drug Res. 30 (1980) 355–357.
[22] R.K. Mirakhur, Drugs 44 (1992) 182–199.
[23] C.L. Hewett, D.S. Savage, J. Chem. Soc. Sect. C (1966) 484–488.
[24] M. Numazava, M. Nagaoko, J. Org. Chem. 47 (1982) 4024–4029.
[25] J. Schmitt, J.J. Panhouse, A. Hallot, P.J. Cornu, P. Comoy, H. Pluchet, Bull. Soc. Chim. France (1963) 807–815.
[26] A.W. Muir, J. Houston, K.L. Green, R.J. Marshall, W.C. Bowman, I.G. Marshall, Br. J. Anaesth. 63 (1989) 400–410.
[27] B. Ginsborg, J. Warriner, Br. J. Pharmacol. Chemother. 15 (1960) 410–411.
[28] O. Arunlakshana, H.O. Schild, Br. J. Pharmacol. Chemother. 14 (1959) 48–58.