Actions of Two Dopamine Derivatives at Adreno- and Cholinoceptors

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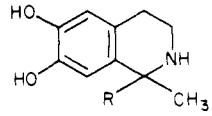
Salsolinol (1) and 6,7-dihydroxy-1,1-dimethyl-1,2,3,4-tetrahydroisoquinoline hydrobromide (2) were synthesized and their effects at adreno- and cholinoceptors investigated both in vivo and in vitro. Both 1 and 2 produced agonist effects at cholinoceptors and α- and β-adrenoceptors. Neuromuscular blocking actions were evident in vitro. Compound 2 exhibited anticholinesterase properties both in vivo and in vitro. These results indicate that dopamine derivatives of this type exhibit not only sympathomimetic activity but also complex actions at cholinoceptors.

The pharmacological activity of certain tetrahydroisoquinolines has long been established.[1] Within the series of 1-substituted 6,7-dihydroxytetrahydroisoquinolines there are several active sympathomimetic amines,[2,3] one of which, trimetoquinol, is a potent bronchodilator.[4-7] To date, all the reported findings with compounds of this series have concerned their activity at adrenoceptors. We have found that, in addition to actions at adrenoceptors, several of these compounds exhibit activity at cholinoceptors. This paper describes the effects of 1-methyl- (1)



1, R = H
2, R = CH_3

and 1,1-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (2).

Chemistry. Salsolinol (1) hydrobromide was synthesized by the method of Craig et al.[2] with minor modifications. 6,7-Dihydroxy-1,1-dimethyl-1,2,3,4-tetrahydroisoquinoline (2) hydrobromide was prepared from one of the intermediates in the synthesis of salsolinol (1).

Thus, 3,4-dihydro-6,7-dimethoxy-1-methylisoquinoline was quaternized with benzyl bromide, the quaternary salt was treated with an excess of methylmagnesium iodide, and the 1,1-dimethyltetrahydroisoquinoline was hydrogenated over 10% palladium on charcoal to effect N-debenzylation. Demethylation with refluxing, constant-boiling hydrobromic acid gave the required product. The hydrochloride salt, prepared by a different route, was used by Gray et al.[8]

Results

(A) In Vivo. In anesthetized cats, both 1 and 2 (0.2–3.0 mg/kg) produced dose-related falls in mean blood pressure, but only 1 caused a fall in heart rate. These effects were antagonized by atropine (1 mg/kg).

In atropinized animals, both 1 and 2 caused dose-related elevations in mean blood pressure that were blocked by phentolamine (2 mg/kg). Compound 1 produced a reduction in the tension and degree of fusion of the incomplete tetanic contractions of the soleus muscle, an effect antagonized by propranolol (0.4 mg/kg). In contrast, 2 induced fasciculations in and augmented (by up to 100% over control) twitches of the skeletal muscle.

(B) In Vitro. In the chick biventer cervicis preparation, 1 (10–200 μg/mL) produced initial twitch augmentation, followed by blockade accompanied by a slowly developing contracture. Responses to exogenous carbachol were unaffected while those to acetylcholine were augmented. The neuromuscular blockade was unable to be reversed by choline, caffeine, physostigmine, or tetanus. Compound 2 (1–10 μg/mL) produced a similar twitch augmentation accompanied by slight contracture. There was, however, no twitch blockade. Concentrations of 2 in excess of 10 μg/mL induced contractions that were rapid in onset, readily maintained, easily reversed by washing, but poorly reproducible.

Table I. Rates of Hydrolysis of Acetylthiocholine by Homogenates of Chick Biventer Cervicis Muscle at 37 °C in the Presence and Absence of 1 and 2[a]

drug	concn μg/mL	concn molar	rate of hydrolysis (μmol min^{-1} g^{-1}) control	rate of hydrolysis (μmol min^{-1} g^{-1}) drug treated
1	333	1.3×10^{-3}	6.82 ± 0.15	7.48 ± 0.14
2	10	3.7×10^{-5}	7.01 ± 0.12	3.55 ± 0.09

[a] Values quoted are the mean ± SE of $n = 4$.

In the guinea pig ileum, 1 rarely produced contraction whereas 2 consistently did so. The resulting log concentration–effect curves for 2 were parallel to those produced by carbachol with similar maxima (Figure 1). The contractions elicited by 2 and carbachol were readily reversed by washing. Atropine antagonized equally the effects of both agonists (Figure 1). Compound 2 was approximately 85 times less potent than carbachol when calculated at the E_{max} = 50 level. Concentrations of hexamethonium that antagonized the action of nicotine were without effect on 2.

In chick biventer cervicis homogenates, 1 in concentrations up to 333 μg/mL (1.3×10^{-3} M; the highest practical concentration) caused no inhibition of the rate of hydrolysis of acetylthiocholine by esterase enzymes (Table I). Compound 2 produced 50% inhibition (I_{50}) of hydrolysis at 10 μg/mL (3.7×10^{-5} M) (Table I). Thus, in comparison with physostigmine that has a reported I_{50} of 2×10^{-8} M,[9] compound 2 was approximately 540 times less potent as an anticholinesterase.

Discussion

In anesthetized cats, both 1 and 2 exhibited agonist activity at muscarinic cholinoceptors and at α- and β-adrenoceptors. The muscle fasciculations and augmentation of soleus muscle twitches induced by 2 are typical characteristics of an anticholinesterase agent. In this capacity, 2 was approximately 200 times less potent than neostigmine. The possibility that the cardiac, blood pressure, and soleus defusion effects were produced indirectly, through release of the appropriate chemical transmitter by 1 and 2, is unlikely but cannot be excluded. Reduction in the tension and degree of fusion of the incomplete tetanic contractions of the soleus muscle is an

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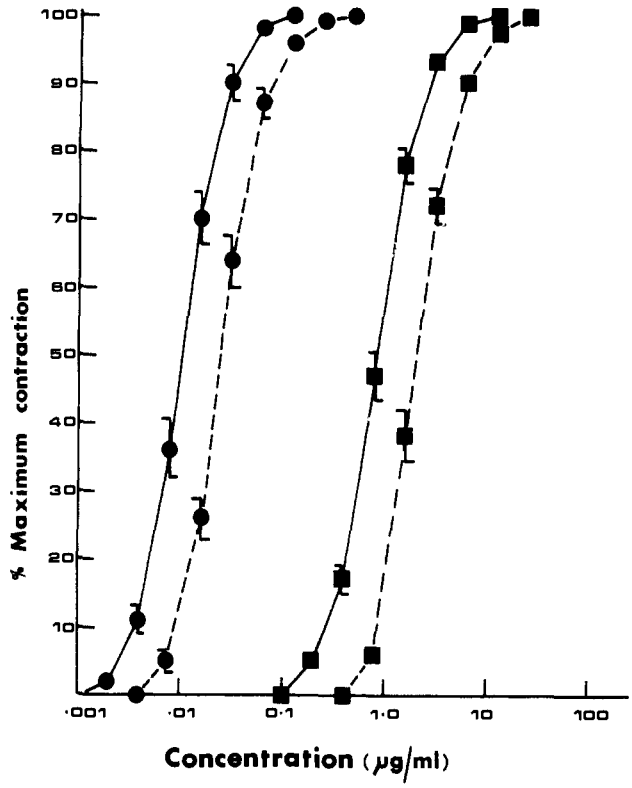

Figure 1. Log concentration-effect curves for carbachol (●) and **2** (■) on the guinea pig isolated ileum preparation. Solid lines represent the control curves before and broken lines the effect after 1×10^{-8} M atropine. The points plotted are the means of at least four separate experiments. Standard errors of the means are illustrated on the symbols ⊢■⊣ and ⊢●⊣.

effect mediated through stimulation of β_2-adrenoceptors.[10] The muscle is known to be sensitive to adrenaline but not noradrenaline;[10,11] hence, any indirect effect would necessitate the release of adrenaline from the adrenal medullae.

In vitro, both compounds possessed activity at cholinoceptors. In the chick biventer cervicis nerve-muscle preparation, both **1** and **2** produced neuromuscular blockade with accompanying contractures. Since the responses to exogenous carbachol were unaffected while those to exogenous acetylcholine were potentiated, it may be concluded that the blockade was of the depolarization type affecting principally the focally innervated fibers. This could be a consequence of anticholinesterase activity, such as was detected in biventer homogenates with **2**. In the smooth muscle of the guinea pig ileum, **1** caused little effect, whereas **2** acted as a full agonist at muscarinic cholinoceptors.

The structural resemblance of the tetrahydroisoquinoline skeleton to dopamine has led to its use in studies of the conformational requirements for agonism at adrenoceptors.[8] In their study, Gray et al.[8] reported that **2** induced contraction of the guinea pig tracheal chain preparation at a concentration of 100 μg/mL. Adrenergic activity was assessed in other preparations over the concentration range 0.01–2000 μg/mL. Our studies have clearly demonstrated the existence of cholinergic effects for salsolinol (**1**) and **2** at concentrations well within the range used by Gray et al.[8] The closely related compound *N*-methyl-1,2,3,4-tetrahydroisoquinoline has also been shown to possess cholinergic activity.[12] The existence of this activity at cholinoceptors clearly complicates the use of such dopamine derivatives in the pursuit of the structural elucidation of adrenoceptors. These actions at cholinoceptors appear to have gone undetected in previous studies.

Experimental Section

(A) Pharmacological Methods. (1) Anesthetized Cat. The experiments were performed on ten adult cats of either sex which were anesthetized by the intraperitoneal injection of a mixture of α-choralose (80 mg/kg) and sodium pentobarbitone (6 mg/kg). The trachea was intubated and the cats were artificially ventilated by positive pressure at a frequency at 27–30 breaths/min and a stroke volume of 13 mL/kg of body weight. The general arterial blood pressure was measured from a cannulated carotid artery by means of a Statham (Model P23AC) pressure transducer. Heart rate was measured by means of a Grass (Model 7P4) tachograph triggered by the general arterial pulse.

The tendon of insertion of a soleus muscle was cut and attached to a Grass (Model FT03) force displacement transducer. Incomplete tetanic contractions of the soleus muscle were evoked by stimulating the peripheral end of the sciatic nerve, which had been dissected out and ligated deep in the thigh, at a frequency of 6–10 Hz for 1 s every 10 s. The method was identical with that described by Bowman and Nott.[10] In some experiments, maximal twitches of the soleus muscle were evoked by stimulating the sciatic nerve at a frequency of 0.1 Hz.

In all but one experiment, the cats were bilaterally vagotomized. All measurements were continuously recorded on a Grass six-channel curvilinear polygraph (Model 7). Drugs dissolved in acid-0.9% (w/v) saline (pH 4.5) were administered through a cannula inserted in either a femoral or brachial vein.

(2) Isolated Chick Biventer Cervicis Nerve Muscle Preparation. Biventer cervicis muscles from chicks aged 3–10 days were set up in Krebs–Henseleit solution at 37 °C and aerated with 5% CO_2 in oxygen. The method was identical with that described by Ginsborg and Warriner.[13]

(3) Isolated Guinea Pig Ileum. The isotonic contractions of segments of guinea pig ileum, set up in Tyrode[14] solution at 32 °C and bubbled with air, in response to several cholinoceptor agonists were recorded on a Washington (Model 400MD/2) pen recorder.

(4) Anticholinesterase Determination. The anticholinesterase activity of the two analogues was assessed by measuring the cholinesterase activity of homogenates of chick biventer cervicis muscle in the presence of each drug according to the colorimetric method of Ellman et al.,[15] as modified by Gandiha et al.[16] The absorbance changes were measured, using an SP 800 spectrophotometer, at a wavelength of 412 nm and a temperature of 32 °C.

(B) Chemical Methods. Melting points were recorded on a Kofler hot-stage apparatus and are uncorrected. IR and 60-MHz NMR spectra were recorded routinely for all steps and were consistent with assigned structures. Elemental analyses were performed by the Chemistry Departments of the Universities of Strathclyde and Manchester, U.K.

6,7-Dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline (1) Hydrobromide (Salsolinol Hydrobromide). 3,4-Dihydro-6,7-dimethoxy-1-methylisoquinoline (1.0 g, 0.005 M) prepared by routine methods[2] was dissolved in EtOH (20 mL), $NaBH_4$ (0.5 g, 0.014 M) was added, and the solution was left overnight. The solution was acidified with dilute HCl, rebasified with 20% NaOH, and extracted with $CHCl_3$ to give a viscous oil, which was dissolved in concentrated HBr (25 mL), refluxed for 4 h, evaporated, and azeotroped with a $CHCl_3$–EtOH mixture, and the residue was crystallized from ethanol–ether to give salsolinol (**1**) hydrobromide as small off-white prisms: mp 195–198 °C (lit.[2] 186–187 °C); MS *m/e* 179 ($C_{10}H_{13}NO_2$, 12%), 178 ($C_{10}H_{12}NO_2$, 18%), 164 ($C_9H_{10}NO_2$, 100%). Anal. ($C_{10}H_{14}NO_2Br$) C, H, N.

6,7-Dihydroxy-1,1-dimethyl-1,2,3,4-tetrahydroisoquinoline (2) Hydrobromide. 3,4-Dihydro-6,7-dimethoxy-1-methylisoquinoline (7.0 g, 0.034 M) prepared as usual was treated with benzyl bromide (6.0 g, 0.035 M) to give as a first crop 6.0 g of off-white solid, which was washed thoroughly with ether containing a little ethanol and then dried in vacuo. This salt (2.0 g, 0.005 M) was added to a solution of MeMgI in ether, which was prepared from MeI (4.0 g) and Mg (0.8 g). After 2 h of stirring, excess reagent was decomposed with NH_4Cl solution, and the aqueous layer was separated and extracted thoroughly with ether. The

combined ether layers were washed with water, dried, and evaporated to give a colorless gum (1.53 g). The gum (1.40 g) was taken up in ethanol (100 mL), and concentrated HCl (2 mL) and 10% Pd on C (0.2 g) were added and hydrogenated overnight at atmospheric pressure. Filtration and evaporation gave a pale-yellow oil (1.1 g). The oil (1.0 g) was dissolved in concentrated HBr (20 mL), and the solution was refluxed for 5 h. It was then evaporated, azeotroped twice with benzene–methanol so as to remove most of the water, and crystallized from 1-butanol to give small pale-gray plates that were washed with 1-butanol and ether and dried thoroughly. The yield at this stage was 0.70 g, but the product contained 1-butanol on crystallization that was removed by recrystallization from ethanol–ether to give small off-white prisms (mp 254–256 °C) containing no butanol (GLC): MS *m/e* 193 ($C_{11}H_{15}NO_2$, 2%), 178 ($C_{10}H_{12}NO_2$, 100%). Anal. ($C_{11}H_{16}NO_2Br$) C, H, N.

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Apparent Bioisosteric Replacement of –S– by NCN: Synthesis of *N*-Cyano-2-aza-*A*-nor-5α-androstan-17β-ol Acetate, an Aza Steroid Androgen

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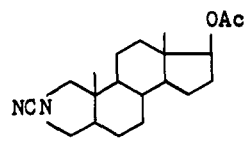
The synthesis of *N*-cyano-2-aza-*A*-nor-5α-androstan-17β-ol acetate is described. Cyclization of 1,4-dibromo-1,4-seco-2,3-bisnor-5α-androstan-17β-ol acetate with benzylamine in the presence of potassium iodide gives the *N*-benzyl-2-aza-*A*-nor steroid. Debenzylation with cyanogen bromide (Von Braun reaction) affords the *N*-cyano-2-aza-*A*-nor steroid, which has androgenic activity slightly weaker than that of the corresponding thia compound. The results indicate that NCN may be substituted for –S– as well as for =S. This compound is the first hormonally active steroid containing nitrogen as a heteroatom in the perhydrocyclopentanophenanthrene nucleus.

In previous papers from this laboratory the synthesis of various androgenic–anabolic heterocyclic steroids has been described.[1-4] Compounds containing sulfur and oxygen as heteroatoms and having one, two, or even three heteroatoms in ring A have been shown to be active. In all cases, we have concluded that it is the steric, rather than the electronic, properties of the ring atoms which are the determinants of pharmacological activity. For one class of heterocyclic steroids, however, the electronic properties may well be of overriding importance. There are the aza steroids in which the basic nitrogen atom could, either by virtue of its high electron density or by the formation of a cationic center, give rise to a biologically inactive compound. This may explain the lack of hormonal activity of the many aza steroids which have been synthesized.

A very recent study[5] on the histamine H_2-receptor antagonist, cimetidine, suggests that cyanoguanidine and thiourea may be classed as true bioisosteres owing to the close similarity of many of their physicochemical properties and the corresponding pharmacological parallelism of these groups with respect to histamine H_2-receptor antagonist activity. In these compounds, =NCN has been substituted for =S. This finding prompted us to synthesize *N*-cyano-2-aza-*A*-nor-5α-androstan-17β-ol acetate (**1**) as a possible bioisostere of thia steroid **2**. In this case, NCN would be substituted for –S–.



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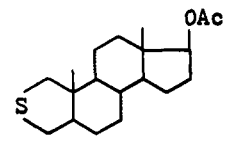

2

Dihydrotestosterone (**3**) (Searle) was acetylated with acetic anhydride in pyridine. Opening of ring A by CrO_3 oxidation gave 17β-acetoxy-2,3-seco-5α-androstane-2,3-dioic acid (**5**),[2,6] which via a modified Hunsdiecker reaction[7,8] afforded dibromide **6**. Dibromide **6** can be cyclized in the presence of a potent nucleophile such as S, Se, or Te with concomitant hydrolysis of the 17-protecting group.[1,2] In the present study, cyclization was unsuccessful with a weaker nucleophile such as benzylamine under the same conditions, but success was attained after addition of KI to the Me_2SO reaction mixture to convert the dibromo compound **6** into the corresponding diiodo compound. Under these conditions, the cyclization with benzylamine proceeded with retention of the 17-

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