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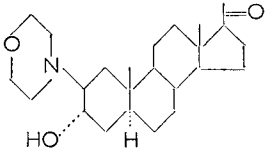
Anticonvulsant and interneuronal blocking activity in some synthetic amino-steroids

SIR,—The electroshock seizure threshold in animals can be raised or lowered by adrenocortical and sex hormones (Woolley & Timiras, 1962a,b and ref. cit.; Woodbury 1958 and ref. cit.). So far only limited success has been achieved in the search for synthetic steroids with potent central nervous system activity, yet devoid of hormonal actions, e.g. Brown & Sarett (1963). Certain amino-esters of 21-hydroxypregnanedione possess general anaesthetic activity (Figdor & others, 1957) and funtumidine (3α-amino-20α-hydroxy-5α-pregnane) is reported to cause tranquillisation (Blanpin & Quevauviller, 1960, and references cited).

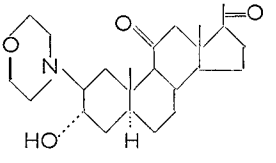
We have investigated a series of amino-steroids for ability to produce loss of righting reflex. In addition, anti-tremorine activity, antagonism to electrically- and chemically-induced seizures and effects on blood pressure, neuromuscular transmission and the crossed extensor reflex in the cat have been examined.

The compounds tested were derivatives of androstane or pregnane in which the amino-radical (amino-, n-propylamino-, n-butylamino-, dimethyl-amino-, diethylamino-, piperidino- and morpholino-) was attached to C-2, C-6 or C-16.

Of the four related compounds, 3α-hydroxy-2β-morpholino-5α-pregnan-20-one (I), 3α-hydroxy-2β-morpholino-5α-pregnane-11, 20-dione (II), 3α-hydroxy-16α-methyl-2β-morpholino-5α-pregnan-20-one (III) and 3α-hydroxy-16α-methyl-2β-morpholino-5α-pregnane-11, 20-dione (IV), the least substituted (I), was the most potent in causing loss of righting reflex.



I



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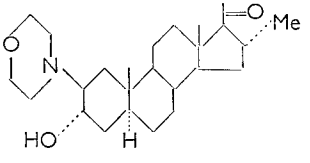
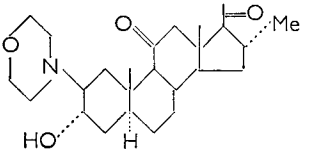


III



IV

Introduction of an 11-keto-group (II) into 3α-hydroxy-2β-morpholino-5α-pregnan-20-one (I) shortens the duration and only slightly increases the degree of this activity; introduction of a 16α-methyl group (III) into (I) lengthens the duration but much reduces the degree of activity. Compound IV, which has both an 11-keto-group and a 16α-methyl group, shows the influence of both substituents; the 16α-methyl group reduces the degree of activity of (II) but increases its duration; the 11-keto group slightly increases the degree of activity of (III) but reduces its duration. The net result relative to (I) is both a diminution of activity (16α-methyl group) and a shortening of the loss of the righting reflex (the effect of the 11-keto group being greater than that of the 16α-methyl group).

Compound V, 3α-hydroxy-2β-morpholino-5α-androstan-17-one, has ED50 and LD50 values for loss of righting reflex much greater than those for (I), although the ED50/LD50 ratio for (V) was the lower.

Compound (VI), 3β,17α-dihydroxy-16β-morpholino-5α-pregnane-11, 20-dione, was the only member of a 16-amino series tested to cause loss of the righting reflex at a dose well below the LD50; both the ED50 and LD50 values were, however, much higher than the corresponding values for the 2β-analogues (Table 1).

TABLE 1. ED50 (LOSS OF RIGHTING REFLEX), DURATION OF EFFECT, LD50 AND ED50/LD50 RATIO IN MICE

Compound number	ED50 (mice) mg/kg (±s.e.)	Dose* mg/kg	Duration of loss of righting reflex in min (±s.e.)	LD50 mg/kg (±s.e.)	Ratio LD50/ED50
I	13 ± 1·63	26	30 ± 5·9	61 ± 2·65	4·69
II	11·8 ± 1·13	24	16·9 ± 1·8	56 ± 1·89	4·75
III	29 ± 0·67	58	37·7 ± 4·8	82 ± 1·42	2·83
IV	19·2 ± 0·84	40	23 ± 1·61	71 ± 2·2	3·64
V	180 ±6·5			340 ± 7·1	1·89
VI	45·5 ± 1·6			107 ± 3·1	2·38
Mephenesin	99 ± 5·7			174 ± 8·2	1·79

* Twice ED50.

Loss of righting reflex is induced by general anaesthetics and neuromuscular and interneuronal blocking agents (O'Dell, 1960). As our compounds did not produce general anaesthesia and had no observed effect on the gastrocnemius muscle–sciatic nerve preparation of the anaesthetised cat, loss of righting reflex was not due to general anaesthesia or neuromuscular blockade. Compounds (I–IV) reduced the crossed extensor reflex in the spinal cat, suggesting that the observed effect was due to interneuronal blockade; a view which is favoured by the observation that, in mice, the pinna reflex disappeared before the corneal reflex (Witkin & others, 1959). The ED50/LD50 ratios of compounds I–VI are higher than that of mephenesin (Table 1).

No anti-electroshock activity was noted after intravenous injection of any compound without simultaneous sedation or a partial loss of righting reflex or both of these.

Compound (I) was the most interesting member of the series in that the intravenous dose required to antagonise the convulsant action of 120 mg/kg of leptazol given intraperitoneally (Berger, 1954) was 3·6 mg/kg, which was one-seventeenth of the LD50 and approximately one-quarter of the ED50 value for loss of the righting reflex. Increasing the time between the injection of (I) and of leptazol from 5 to 20 min, led to a twofold increase in the PD50 (protective) value (6·3 mg/kg) which, nevertheless, was still lower than the ED50 value for loss of righting reflex. A search of the literature has so far revealed no other synthetic steroid possessing marked anti-leptazol activity.

None of the six above-mentioned compounds, on intravenous administration, antagonised tremorine-induced tremors.

For optimum activity in producing loss of righting reflex and anti-leptazol activity, the following requirements appear to be the necessary minimum—(*a*) a pregnane nucleus, (*b*) a 2β-morpholino-group, (*c*) a 3α-hydroxyl group.

Although the amino-esters of 21-hydroxypregnanedione of Figdor & his colleagues (1957) had general anaesthetic activity and the compounds (I–VI) we have investigated possessed loss-of-righting-reflex and anti-leptazol activities, there is agreement on two points: (*a*) the morpholino-substituted steroid was the most active and (*b*) nuclear substitution decreased potency.

A further compound, (VII), 3β-acetoxy-5α-hydroxy-6β-morpholino-5α-pregnan-20-one, produced convulsions in mice and these superficially resembled leptazol convulsions.

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A sensitive preparation for the assay of 5-hydroxytryptamine

SIR,—The crop of the young chick contracts strongly in response to minute amounts of 5-hydroxytryptamine (5-HT) and has been used as a sensitive assay preparation.

Chicks (Silver Link) up to 7 days old were starved overnight to empty the crop and then killed with ether. The crop was removed and placed in a petri-dish containing Krebs-Henseleit solution (g/litre: NaCl 6·95, KCl 0·34, $CaCl_2$ 0·28, KH_2PO_4 0·162, $MgSO_4$ 0·294, $NaHCO_3$ 2·1 and dextrose 2) at room temperature. It was opened by a longitudinal cut in the wall of the attached portion of oesophagus and a strip of tissue about 3 mm wide was then cut transversely from the middle of the opened crop. The strip was suspended in a Perspex bath of just over 1 ml capacity containing Krebs solution at room temperature (20-23°) which was bubbled slowly with 5% carbon dioxide in oxygen. One end of the strip of crop was attached to an isotonic frontal writing lever loaded with 4 g and magnifying the contractions 12 times. An interval of 30-60 min was