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Short Research Article

Labelling of steroid 3-O-sulfates by tritium and their binding to guinea pig cortical cell membranes†

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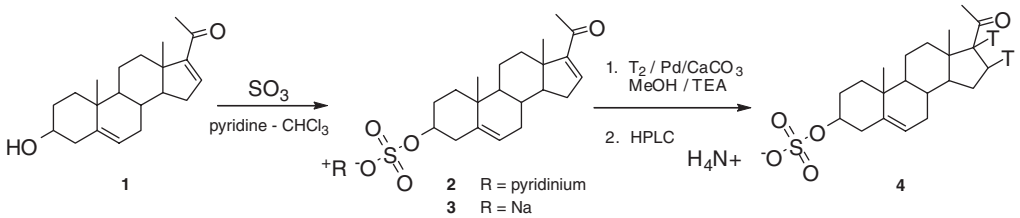
Introduction

Steroids are known to modulate the activity of several membrane receptors. Especially pregnenolone sulfate is supposed to be bound to NMDA receptors and to allosterically modulate their activity. To study the receptors, the tritiated steroid 3-O-sulfates with high specific activity had to be prepared (Scheme 1,2).



Scheme 1

Results and discussion

Synthesis of labelled compounds

The 3-β-hydroxy-pregn-5,16-dien-20-one 3-O-sulfate pyridinium salt **2** was prepared from diene **1** and this was converted to sodium salt **3**. Sodium salt **3** was tritiated in methanol–triethylamine 1:1 (v/v) mixture with 5% $PdO/CaCO_3$ as catalyst under carrier-free tritium gas. The desired ammonium salt **4** was separated from the mixture (composed of starting compound **3**, product **4** and fully saturated byproduct) by radio-HPLC (the mobile phase contained 25 mM of ammonium formate). The specific activity was 1.28 TBq/mmol (47.5 Ci/mmol) and the radiochemical purity was better than 99%. The stock solution of [^{3}H]pregnenolone sulfate ammonium salt **4** in DMSO (1.4 MBq/ml) was kept in dark at room temperature. After 71 days, the radiochemical purity was still 96.6%.

Analogously the 3-α-hydroxy-[^{3}H]pregnanolone 3-O-sulfate ammonium salt **6** was prepared by tritiation of 3-α-hydroxy-pregn-16-en-20-one 3-O-sulfate sodium salt **5**. The specific activity was 1.08 TBq/mmol (40 Ci/mmol) and radiochemical purity better than 99%.

The stability of tritium on the C-17 during the synthesis and purification of **4** and **6** was proofed by deuteration of **5** under the same conditions and analysis of its ^{1}H and ^{13}C NMR spectra.

Binding to cell membranes

The binding of [^{3}H]pregnenolone sulfate **4** to guinea pig cortex and hypothalamus membranes was not displaceable by non-labelled pregnenolone sulfate. Moreover, a kind of paradoxical binding was observed, i.e. the amount of activity bound to the membranes

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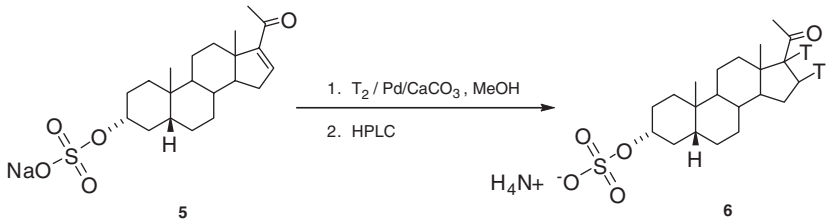

Scheme 2

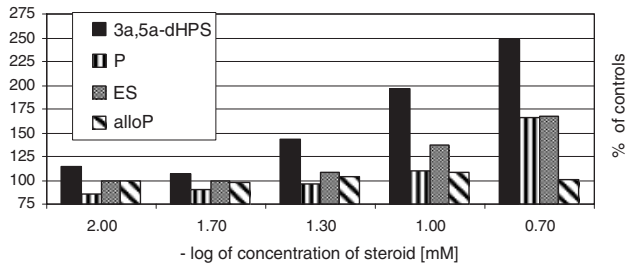

Figure 1 Binding of [^{3}H]pregnenolone sulfate on the cortex membranes of guinea pig in the presence of various steriods.

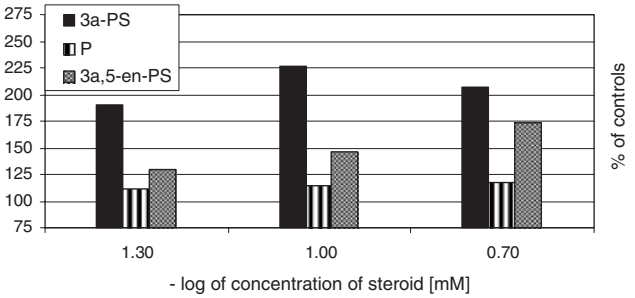

Figure 2 Binding of 3-alfa-[^{3}H]pregnanolone sulfate on the cortex membranes of guinea pig in the presence of various steriods.

increased with increasing the concentration of unlabelled compound. To verify this observation also the other steroids were tested on their ability of paradoxical enhancement of binding of labelled **4** to the membranes. As seen from Figure 1 the effect was structure dependent. The following steroids were tested: 3α,5α-pregn-16-en-olon 3-O-sulfate (3a,5a-dHPS); pregnenolon (P); estradiol 3-O-sulfate (ES); allopregnanolon (alloP) and 3-α-pregnenolone 3-O-sulfate (3a,5-en-PS). The control value is the activity bound to membranes in the absence of any non-labelled steroid.

The 3-α-hydroxy-[^{3}H]pregnanolone 3-O-sulfate **6** was tested for its binding capacity against guinea pig cortex membranes and mice liver membranes (a negative control). As for [^{3}H]pregnenolone sulfate **4** only the paradoxical enhancement of the radioactivity bound to guinea pig membranes was observed (Figure 2). The effect was highest for non-labelled 3-α-hydroxy-pregnanolone 3-O-sulfate (3a-PS) itself. The binding of **6** to mice liver membranes even in the presence of non-labelled steroids was substantially lower.

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