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SHORT COMMUNICATION

FAILURE TO INTERPRET THE BEHAVIOURAL EFFECT OF CORTISOL-21-SULPHATE BY STEROID-RECEPTOR INTERACTION

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SUMMARY

To elucidate whether the behavioural action of cortisol 21-sulphate (F-21-S) on the central nervous system (CNS) can be explained on the basis of steroid-receptor binding, the effect of F-21-S on corticosterone (B) binding to cytosol protein was investigated. Rat brain cytosol was prepared essentially according to the method of Roth. Approximately 1.25 mg of cytosol protein was incubated with 1 pmol (10^5 d.p.m.) of [^{3}H]-B in 500 μl of assay mixture at 0°C for 4 h with and without cold B or cold F-21-S. Dissociation constant (K_D) and binding maximum (B_{max}) of B-rat brain cytosol receptor protein(s) was 4.0×10^{-9} M and 2.9×10^{-13} mol per mg cytosol protein, respectively. F-21-S did not exhibit on this binding as lower concentration than 200 nM. The results suggest mechanism(s) other than steroid receptor binding for the behavioural action of F-21-S.

Glucocorticoids are known to affect conditioned behaviour in rats[1]. McEwen and Wallach[2] have recently shown specific accumulation of [^{3}H]-B in both the septum and hippocampus of rat brain. Moreover, it has been suggested that the binding of B to cytosol receptors and its cell nuclear uptake in the areas is of primary importance for the bahavioural effect in rats. Interestingly, Miyabo *et al.*[3] have demonstrated that F-21-S stimulates exploratory behaviour in rats, despite the fact that the conjugated steroids administered had no biophysical effect.

Therefore, it is worthwhile to investigate whether the effect of F-21-S to the CNS can be explained on the basis of steroid receptor binding as the first step necessary for cellular responses to hormones. The present study was designed to examine this question. Additionally, for a purpose of comparison with the other inactive steroids, the binding affinities with cytosol receptor of rat brain of 14 non-conjugated steroids, including 4 cortisol metabolites, were examined.

1,2-[^{3}H]-B (41 Ci/mmol) was purchased from the New England Nuclear Co. (Boston, MA). Authentic steroids were obtained from Ikapharm Co. (Israel) and F-21-S was supplied from Schering Co. (Germany).

Seven days after the adrenalectomy of male rats of Wister-strain ranging in weight from 200–250 g were killed by decapitation at 9:00 A.M. Rat brain cytosol protein was obtained according to the method of Roth[4]. 0.25 ml Of cytosol solution with 0.01 M Tris–HCl (pH 7.4 at 0°C) containing 1.25 mg of protein was incubated with [^{3}H]-B (100 μl) at a final concentration of 2×10^{-9} M in the absence or presence of an excessive dose of non-radioactives, the maximum concentration was 5000 fold of radioactive B, at 0°C for 4 h in the total volume of 500 μl. The separation of the unbound steroid from the bound [^{3}H]-B with the cytosol was carried out after incubation by adding a 0.1 mg/ml suspension of freshly activated charcoal (Norit A). After centrifugation at 1500 *g* for 20 min, 200 μl of supernatant in 10 ml of scintillation fluid containing liquifluor, 4.2%; Triton X, 33.4%, and toluene, 62.4% were counted for radioactivity with automatic external standardization to measure quenching.

According to Scatchard analysis[5], the B_{max} was 2.9×10^{-13} mol/mg cytosol protein and the K_D was 4.0×10^{-9} M. The competitive effect of several steroids on [^{3}H]-B binding to rat brain cytosol receptor(s) were shown in Table 1 with the concentration of each steroid required for 50% displacement of the specific binding to the cytosol of [^{3}H]-B. A steroid such as dehydroisoandrosterone, both reduced and oxidixed metabolites of cortisol, and F-21-S had no competition with [^{3}H]-B in binding to receptor protein.

In general, the reduced or oxidized metabolites of biologically active steroids are no longer biologically active and appear to lose the ability to bind to receptor(s). In the present study it was shown that the THF, cortol, cortolon and 11-etiocholanolone which are metabolites of cortisol had no affinity to the cytosol receptor of rat brain.

Concerning the behavioural effect of conjugated steroids, Miyabo *et al.*[3] have demonstrated that the i.m. administration of F-21-S to young male rats exerted significant stimulatory effects on the animals exploratory behaviour. They suggested that the effects are probably due to a direct action of F-21-S on the CNS rather than its effects on

Table 1. Concentrations (nM) of various steroids required for 50% displacement of [^{3}H]-corticosterone from the cytosol receptors

Steroid	Concentration (nM)
Corticosterone (B)	9.1
Aldosterone	5.5
β-Methasone	8.0
Desoxycorticosterone	11.2
Cortisol (F)	30.0
Progesterone	98.4
Dexamethasone	60.8
17-OH-Progesterone	328.4
Testosterone	1210.0
Cortisol-21-sulfate (F-21-S)	Infinite
α-Cortol	Infinite
α-Cortolone	Infinite
11-OH-Etiocholanolone	Infinite
Tetrahydrocortisol (THF)	Infinite
Dehydroepiandrosterone	Infinite

intermediary metabolism or negative feedback on pituitary ACTH release. If the effects of the conjugate on behavioural actions in rats are caused by the same mechanism as that of free glucocorticoid, the binding of the conjugate to cytosol receptor has to be demonstrated as the first step of hormone action. However, in the present study the author failed to demonstrate this possibility: F-21-S did not displace the binding of [^{3}H]-B to cytosol receptor protein obtained from rat brain. Therefore, the author thinks that behavioural effects of a pharmacological dose of F-21-S in rats as observed by Miyabo *et al.* is due to influences of the steroid on brain independent of steroid receptor interaction.

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