Relationship Between R_m Values and Protein Binding of Corticosteroids and Androgens

The usefulness of R_m values for structure/activity studies in a series of testosterone esters was previously pointed out[1,2]. The purpose of the present work was to show a relationship between R_m values and albumin-binding of corticosteroids and androgens. The R_m values of the test compounds were determined by means of a reversed-phase TLC method, the details of which have already been described[1].

The mobile phase consisted of water in various mixture (v/v) with acetone. The stationary phase was represented by a Silica Gel G layer impregnated with Silicone oil. In the case of corticosteroids, the acetone concentrations in the mobile phase ranged from 16 to 23.5%; in that of androgens from 24 to 52%. Because of a linear relationship between R_m values and acetone concentrations in the mobile phase[1], it was possible to calculate, for each compound, a theoretical R_m value corresponding to a 0% acetone concentration (Table).

In the Table are reported some data of CHEN et al.[3] regarding the protein-binding of the test compounds. Log BR indicates the logarithm of the percentage ultrafilterable fraction. Since the ultrafilterable fraction is inversely related to the bound fraction, steroids with high log BR values are bound to the least extent. The best rationalization of the relationship between log BR and R_m values is provided by Eq. 1, with a correlation coefficient of 0.964.

$$\log BR = 2.290 - 0.672\, Rm \quad (n = 9,\ r = 0.964,\ s = 0.094) \qquad (1)$$

The negative sign associated with the R_m term shows that protein-binding increases with the R_m values. Since higher R_m values indicate compounds more lipophilic, protein-binding depends linearly on the lipophilic character of molecules.

This result is in agreement with those of HANSCH et al.[4,5], FUJITA et al.[6] and BIRD et al.[7], who showed that protein-binding depends mainly on lipophilicity in several series of drugs. In the case of acids or bases, also the electronic character of molecules can play an important role in protein-binding. KLOTZ et al.[8] showed that the pK_a values, which can be considered as an expression of the electronic character of molecules, were inversely related with the protein-binding of sulfonamides. FUJITA[9] showed that uncoupling activity of phenols is mainly dependent on their pK_a values. However, in a series of uncharged molecules, such as steroid compounds, the most important factor seems to be the lipophilic character, which can be usefully expressed by the chromatographic R_m values. The TLC technique has its major limit in the adsorption phenomena which can affect the distribution of the compounds between the polar phase and the non-polar one. Therefore the direct measurement of the partition coefficient seems still to provide the most reliable results. However, the advantages of the chromatographic technique, as pointed out by BOYCE and MILBORROW[10], are noteworthy. The method is rapid and simple, and requires a very small amount of material; this does not need to be very pure because impurities are separated during the migration. The detection of spots by unspecific methods avoids the need for specific quantitative analytical procedures. Finally, in an earlier paper[1] a very good correlation was shown between the R_m values of testosterone esters and their π values obtained from the partition data in an octanol/water system.

Riassunto. Un metodo cromatografico su strato sottile a fasi invertite ha permesso di ottenere valori R_m altamente correlati con il legame con l'albumina in due serie di corticosteroidi e androgeni.

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Albumin binding and R_m values of various steroids

Steroid	log BR[3]	R_m
Androsterone	0.74	2.391
Deoxycorticosterone	0.90	2.057
Dehydroisoandrosterone	0.95	1.790
Corticosterone	1.25	1.447
Dehydroandrosterone	1.28	1.607
Cortisone	1.50	1.121
Hydrocortisone	1.49	1.214
11 β Hydroxyandrostenedione	1.60	0.940
Prednisolone	1.69	1.141

The log BR values indicate the logarithm of the percentage ultrafilterable fraction.

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Failure of Hemicholinium-3 to Inhibit the Uptake of ^{3}H-Choline in Mouse Brain in vivo

Tritium-labelled choline and acetylcholine have been detected in mouse brain almost immediately after i.v. injection of ^{3}H-choline of high specific activity[1]. Thus choline is able to pass the blood brain barrier in spite of being a polar quaternary ammonium compound. This uptake of choline may therefore be due to an active mechanism of the same type as that operating in the cholinergic nerve terminals. Since the neuron uptake is inhibited by hemicholinium-3[2] and troxopyrrolidinium[3], we considered it of interest to investigate the effect of these compounds on the uptake of ^{3}H-choline into mouse brain in vivo.

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Effects of hemicholinium-3 dibromide and troxopyrrolidium chloride on the uptake of ^{3}H-choline in mouse brain in vivo

Experiment number	Treatment	Time before ^{3}H-choline[b] (min)	Dose (mg/kg i.p.)	n	^{3}H-choline uptake (nmoles/g ± S.E.M.)
1	Saline	30	–	6	3.2 ± 0.2
	Troxopyrrolidinium chloride	30	12.5	5	3.5 ± 0.1[a]
2	Saline	30	–	7	3.3 ± 0.6
	Hemicholinium-3 dibromide	30	0.1	8	3.6 ± 0.4[a]
3	Saline	15	–	10	4.4 ± 0.4
	Hemicholinium-3 dibromide	15	0.1	5	4.9 ± 0.6[a]
	Hemicholinium-3 dibromide	15	0.2	5	4.5 ± 0.7[a]

[a] $p > 0.05$ in comparison with the controls (Student's t-test); [b] 25 µmoles/kg i.v. of choline-methyl-^{3}H chloride were injected and the mice killed exactly 30 sec later.

The compounds (hemicholinium-3 dibromide and troxopyrrolidinium chloride) were injected i.p. at various times before the i.v. injection of 25 µmoles/kg of choline methyl-^{3}H chloride (Amersham, England, specific activity 500 mCi/mmole diluted to 4 mCi/nmole with inactive choline) to male mice weighing 20–24 g. The mice were killed by decapitation exactly 30 sec after the injection. The brains were weighed and disintegrated in 3 ml of Soluene-100 TM (Packard) at room temperature. When completely dissolved, 15 ml of scintillation liquid (toluene PPO-POPOP) were added and the radioactivity was measured. Standards containing disintegrated brains were measured simultaneously. The amount of radioactivity was expressed as nmoles of ^{3}H-choline taken up per g brain tissue.

The results are summarized in the Table. About 0.3% of the total amount of the ^{3}H-choline was found in the brain 30 sec after the injection. This is somewhat less than the amount (0.45%) reported by SCHUBERTH et al.[1]. Neither hemicholinium-3 nor troxopyrrolidinium had any inhibitory effect on the uptake of ^{3}H-choline in the brain, even when administered in high doses. Thus, the uptake of choline through the blood brain barrier does not seem to be determined by the same mechanism as that operating in the cell membranes of cholinergic neurons.

Zusammenfassung. Es wird die Wirkung von Hemicholinium-3-dibromid und Troxopyrrolidinium chlorid auf die Aufnahme von [^{3}H]Cholin im Gehirn der Maus studiert. Beide Substanzen hatten keine Wirkung, und es ist wahrscheinlich, dass der Mechanismus der Cholinaufnahme in das Gehirn vom dem der Cholinaufnahme in die cholinergischen Nervendigungen verschieden ist.

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Eine bisher unbekannte endokrine Drüse im Kopf von *Scutigera coleoptrata* L. (Chilopoda, Notostigmophora)

Bei Untersuchungen über das endokrine System von *Scutigera coleoptrata* wurden paarige, bisher nicht beschriebene Drüsen innerer Sekretion gefunden. Sie liegen kaudal der Protocerebralloben seitlich und etwas dorsal vom mittleren Vorderdarm in einem engen Hämolymphsinus zwischen Fettkörperlappen und Muskeln und sind angenähert spindelförmig (Figur 1). Bei adulten Tieren haben sie eine durchschnittliche Länge von 150 µm bei einer grössten Dicke von ca. 75 µm. Sie sollen vorläufig «endokrine Kopfdrüsen» genannt werden.

Die Längsachsen der Drüsen sind ungefähr dorsoventral ausgerichtet. Im Querschnitt hat jede Drüse die Form eines flachen U, das nach der Medianen des Körpers geöffnet ist. Die Lappen, die den beiden Schenkeln des U entsprechen, umfassen ein Blutgefäss, das der Drüse als Aufhängevorrichtung dient (Figur 1). Die Oberfläche der Drüse erscheint unregelmässig gefurcht.

Elektronenmikroskopische Aufnahmen zeigen eine sehr grosse Ähnlichkeit der endokrinen Kopfdrüsen mit der kürzlich beschriebenen unpaaren Kragendrüse des Diplopoden *Polyxenus lagurus*[1,2]. Ihre Zellen grenzen sich gegen das umgebende Hämocoel durch die Basalmembran ab (Figur 2, Bm). Die Basen der Zellen bilden viele fingerförmige Fortsätze aus, die der Basalmembran wie die Pedicellen von Podocyten aufsitzen. Das Bild von Podocyten wird dadurch vervollständigt, dass ab und zu zwischen benachbarten Pedicellen Diaphragmata angetroffen werden (Figur 2). Die Zellen überlappen sich stark. Deshalb wird die Kopfdrüse von den vielen Interzellularspalten zwischen ihren lateralen Cytoplasmamembranen nach allen Richtungen durchzogen (Figuren 2–4, Is). Die Interzellularspalten sind in ihrem Verlauf unterschiedlich weit. An ihren Membranen kommt es vor allem im basalen Zellbereich zu zahlreichen cytotischen Vesikulationen (Figuren 3 und 4, V). Auch die abgeschnürten Vesikel im Zellinnern lassen die dreischichtige Struktur der Elementarmembran erkennen (Figur 4). Im Innern der Vesikel ist die fädige Glykokalyx weiterhin sichtbar. Der cytoplasmatischen Seite der Vesikelmembran sitzen dichte Projektionen an, wie sie für «coated vesicles» charakteristisch sind. Zwischen den Vesikeln ist das Cytoplasma mit vielen elektronendichten tubulären Strukturen angefüllt (Figuren 2 und 3, T). Manchmal umschliessen diese eine transparente Fläche auf dem Schnitt kreisförmig (Figur 2, *). Selten sitzen sie mit einem ihrer Enden direkt einem der cytotischen Vesikel an. Vesikel und tubuläre Strukturen kommen auch im Zentrum der Zelle vor, dort jedoch nicht so ausschliesslich. Dafür findet man hier neben zahlreichen cristären Mitochondrien (Figuren 2 und 4, M), die gross sind und

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