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Production of optically Specific Antiserum to *l*-Pentazocine

In order to obtain the optically specific antiserum used for radioimmunoassay of *l*-pentazocine a hapten-carrier conjugate was prepared from *l*-pentazocine 2′-carboxymethyl ether by coupling with bovine serum albumin employing the mixed anhydride technique. The antiserum elicited in the rabbit by immunization with this antigen was found to be highly specific to *l*-pentazocine.

Keywords——*dl*-pentazocine; analgetic drug; *l*-pentazocine-BSA conjugate; anti-*l*-pentazocine antiserum; ^{3}H-*l*-dihydropentazocine; cross-reactivity; radioimmunoassay

dl-Pentazocine (*cis*-2-(3,3-dimethylallyl)-2′-hydroxy-5,9-dimethyl-6,7-benzomorphan) (Ia) is a non-narcotic analgetic drug extensively used. The pharmacological activity is known to be approximately twenty times as great with *l*-pentazocine (Ic) as with its enantiomer (Ib).[1] The pharmacokinetic studies of this drug require the development of a new method for separatory quantitation of *l*-pentazocine in blood plasma. The mass fragmentographic determination of some enantiomeric drugs in biological fluids by means of a deuterium labeling technique has recently been reported.[2–4] We have attempted to develop a radioimmunoassay for *l*-pentazocine having minimal cross-reactivity. In this communication we report the specificity of antiserum raised against *l*-pentazocine when *l*-pentazocine 2′-carboxymethyl ether-bovine serum albumin (BSA) conjugate (Ie) was used as an immunogen.

It was decided to conjugate *l*-pentazocine to the carrier through the phenolic group, thus allowing the characteristic asymmetric center and the N-dimethylallyl group to exert their maximum effects as antigenic determinants. An initial project was therefore directed to the preparation of *l*-pentazocine of optically high purity. *dl*-Nor base (*cis*-2′-hydroxy-5,9-dimethyl-6,7-benzomorphan) (IIa) was resolved by crystallizing the *d*-tartrate from the aqueous solution. This procedure was repeated several times to provide *l*-nor base (IIb), mp 220—230°, $[\alpha]_D^{25}$ −70° (c=1 in EtOH), in the pure state. Being refluxed with dimethylallyl bromide in dimethylformamide, *l*-nor base was readily transformed into *l*-pentazocine, mp 164—170°, $[\alpha]_D^{25}$ −131° (c=1 in $CHCl_3$). Condensation with monochloroacetic acid afforded the 2′-carboxymethyl ether (Id) as an oily product. IR ν_{max}^{KBr} cm^{-1}: 1610 (COO^-), NMR ($CDCl_3$) δ: 0.83 (3H, d, J=6 Hz, 9-CH_3), 1.33 (3H, s, 5-CH_3), 1.70 (6H, d, J=4 Hz, $>C=C(CH_3)_2$), 4.48 (2H, s, $-OCH_2CO-$), 5.38 (1H, t, J=12 Hz, $-CH=C<$), 6.88 (3H, m, aromatic H), $[\alpha]_D^{25}$ −59° (c=0.18 in EtOH). This hapten was covalently linked through its carboxyl group to BSA by the mixed anhydride method using tri-*n*-butylamine and isobutyl chloroformate. As judged from the ultraviolet absorption at 280 nm it was proved that thirty-five moles of hapten were bound to each mole of BSA.

The hapten-carrier conjugate was dissolved in sterile isotonic saline, emulsified with Freund's complete adjuvant, and injected into rabbits subcutaneously at the multiple sites over the scapulae and in the thighs. This procedure was repeated at intervals of two weeks for two months and then once a month. After four months the serum sample obtained from the immunized rabbit showed significantly increased binding activity to *l*-pentazocine. Using ^{3}H-*l*-dihydropentazocine (IIc), prepared from *l*-pentazocine by catalytic hydrogenation with $^3H_2$ gas over palladium-on-charcoal, as a labeled antigen, a standard curve for *l*-pentazocine was constructed with 1: 30000 dilution of the rabbit serum.

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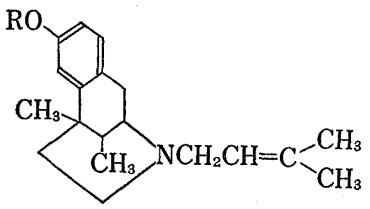
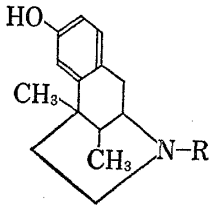
The specificity of antiserum was assessed by testing the ability of some compounds closely related to *l*-pentazocine to compete for binding site on the antibody. The results of cross-reaction studies with anti-*l*-pentazocine antiserum are collected in Table I. It is evident from the data that the antiserum obtained is highly specific for *l*-pentazocine exhibiting no significant cross-reaction with *d*-pentazocine and principal metabolites, *dl-trans*-alcohol (IId) and *dl-trans*-acid (IIe).[5-7] *dl*-Pentazocine showed the cross-reactivity of 51.2%, which seems to be a reasonable value in view of the above data.

It is to be noted that immunization with an optically active hapten-protein conjugate produces the highly specific antibody whose availability enables us to determine a trace amount of the optical antigen without being influenced by its enantiomer and related compounds by means of radioimmunoassay.



Ia: *dl*, R=H
Ib: *d*, R=H
Ic: *l*, R=H
Id: *l*, R=CH_2COOH
Ie: *l*, R=$CH_2CONHBSA$



IIa: *dl*, R=H
IIb: *l*, R=H
IIc: *l*, R=$CH_2CH(^3H)-C(^3H)<^{CH_3}_{CH_3}$
IId: *dl*, R=$CH_2>C=C<^{H}_{CH_2OH}^{CH_3}$
IIe: *dl*, R=$CH_2>C=C<^{H}_{COOH}^{CH_3}$

Chart 1

TABLE I. Per Cent Cross-Reaction of Anti-*l*-Pentazocine Antiserum with Closely Related Compounds to *l*-Pentazocine

Compound	Cross-reaction (%)
l-Pentazocine (Ic)	100
dl-Pentazocine (Ia)	51.2
d-Pentazocine (Ib)	0.084
dl-trans-Alcohol (IId)	0.63
dl-trans-Acid (IIe)	<0.01

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