Isolated Adrenal Cells: The Partial Agonists [Trp(Nps)^9]ACTH$_{1-39}$[1] and [Trp(Nps)^9]ACTH$_{1-24}$ (Nitrophenyl Sulfenyl Derivatives of ACTH)[2] (36332)

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Ramachandran and Lee (1) reported that the modification of the tryptophan residue of ACTH by reaction with o-nitrophenyl sulfenyl (Nps) chloride resulted in a total loss of lipolytic activity of the hormone as tested on isolated fat cells of the rat; furthermore, the Nps derivative completely inhibited the lipolytic activity of ACTH on adipocytes when the ACTH:inhibitor ratio was 1:25.

When tested in the isolated rat adrenal cell system of Sayers *et al.* (2), [Trp(Nps)^9]ACTH$_{1-39}$ acts as a partial agonist, that is, the compound 1) stimulates corticosterone production, 2) induces a maximum biological response less than that of the natural agonist, ACTH$_{1-39}$ and 3) inhibits ACTH$_{1-39}$ at appropriate combinations of doses. These observations emphasize the importance of tryptophan in the processes involved in excitation of receptor by the ACTH molecule.

Materials and Methods. [Trp(Nps)^9]ACTH$_{1-39}$ and [Trp(Nps)^9]ACTH$_{1-24}$ were prepared by the method of Scoffone *et al.* (3) and were generously provided by Dr. Rittel of CIBA-GEIGY AG, Basel, Switzerland. ACTH$_{1-39}$ was the 3rd International Standard [1 μU $\equiv$ 10 pg $\equiv$ 2.2 fmoles (fmole = femtomole = 10^{-15} mole)] and ACTH$_{1-24}$ (Cortrosyn) was provided by Dr. H. Strade, Organon Ltd., West Orange, NJ.

ACTH$_{1-24}$ and [Trp(Nps)^9]ACTH$_{1-24}$ exhibited R_f's of 0.40 and 0.54, respectively, in ascending paper chromatography [Whatman No. 1 in butanol:acetic acid:pyridine:water (30:6:24:20)]. A total of 4 mg of [Trp(Nps)^9]ACTH$_{1-24}$ was applied in 200 μg quantities on 9 in. wide paper. The chromatogram was developed for 14–15 hr. It was then air dried in a hood. The region corresponding to the R_f value for [Trp(Nps)^9]ACTH$_{1-24}$ was cut from the chromatogram, eluted with 10% acetic acid and lyophilized. The lyophilized derivative was rechromatographed by the same procedure.

A suspension of cells of the rat adrenal cortex was prepared by the trypsin method of Sayers *et al.* (2). The cells were harvested as a pellet by centrifugation of the dispersate and the pellet was resuspended in 60 ml of Krebs–Ringer bicarbonate buffer containing calcium, 7.65 m*M*, glucose, 0.2%, bovine serum albumin, 0.1%, and Lima Bean trypsin inhibitor (Worthington Biochemical Corp.), 0.1%. Aliquots of the suspension, 0.9 ml in volume, together with 0.1 ml of vehicle or with 0.1 ml of vehicle to which ACTH had been added, were incubated in an atmosphere of 95% O_2:5% CO_2 at 37°. After 60-min incubation, methylene chloride was added and an aliquot of the methylene chloride was analyzed for corticosterone (4).

Results and Discussion. ACTH$_{1-39}$ and [Trp(Nps)^9]ACTH$_{1-39}$ were added alone

[1] The following abbreviations of amino acids and peptides are used [IUPAC-IUB Commission on Biochemical Nomenclature, *Eur. J. Biochem.* 1 (1967) 375]: ACTH$_{1-39}$ = porcine adrenocorticotropic hormone; [Trp(Nps)^9]ACTH$_{1-39}$ = [9-tryptophan(o-nitrophenyl sulfenyl)]-porcine adrenocorticotropic hormone; ACTH$_{1-24}$ = corticotropin-(1-24)-tetracosapeptide; [Trp(Nps)^9]ACTH$_{1-24}$ = [9-tryptophan (o-nitrophenyl sulfenyl)]-corticotropin-(1-24)-tetracosapeptide.

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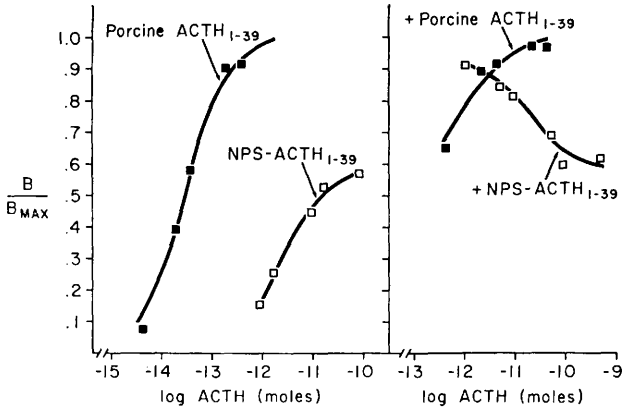

FIG. 1. LDR curves for $ACTH_{1-39}$ (■—■) and for $[Trp(Nps)^9]ACTH_{1-39}$ (□—□) added alone (left panel) and LDR curves for combinations (right panel). In the right panel increasing quantities of $[Trp(Nps)^9]ACTH_{1-39}$, presented on the abscissa, were added to a fixed dose of 2.2×10^{-13} mole $ACTH_{1-39}$ (□—□); increasing quantities of $ACTH_{1-39}$, presented on the abscissa, were added to a fixed dose of 1×10^{-10} mole $[Trp(Nps)^9]ACTH_{1-39}$ (■—■). The points are the observed values of B/B_{max}; the lines are the best fit curves (Eq. 1). Value of B_{max} for this suspension of cells equalled 3.09×10^{-9} moles corticosterone/60 min.

and in combination to aliquots of a single suspension of isolated adrenal cells. The comparison of the log dose–response (LDR) curves for $ACTH_{1-39}$ and $[Trp(Nps)^9]ACTH_{1-39}$ alone suggests that the derivative is a partial agonist (left panel, Fig. 1). The LDR curve for $ACTH_{1-39}$ may be expressed as $B/B_{max} = \alpha A/(A + A_{50})$ where B is the rate of corticosterone production, B_{max} is the maximum rate of corticosterone production, α is the intrinsic activity (5) of $ACTH_{1-39}$, A is the dose of $ACTH_{1-39}$ and A_{50} is the dose of $ACTH_{1-39}$ required to induce $1/2\ \alpha B_{max}$. $ACTH_{1-39}$ is assumed to have an α value of 1, that is, $ACTH_{1-39}$ has the capacity to achieve maximum excitation of the receptor and is defined as an agonist. The LDR for $[Trp(Nps)^9]ACTH_{1-39}$ may be expressed as $B/B_{max} = \beta P/(P + P_{50})$ where β is the intrinsic activity of $[Trp(Nps)^9]ACTH_{1-39}$, P is the dose of the derivative and P_{50} is the dose required to induce $1/2\ \beta B_{max}$. Estimates of A_{50}, P_{50} and β were obtained by nonlinear least square regression analysis with the aid of a computer (6). Estimates of apparent dissociation constants A_{50} and P_{50} for $ACTH_{1-39}$ and $[Trp(Nps)^9]ACTH_{1-39}$ acting alone were 16.7 fmoles and 2890 fmoles, respectively; β for the derivative, 0.62.

Further support for the conclusion that the derivative is a partial agonist is derived from the results of combinations. When increasing quantities of $[Trp(Nps)^9]ACTH_{1-39}$ were combined with a fixed quantity of $ACTH_{1-39}$, the response was reduced from αB_{max} to βB_{max} (right panel, Fig. 1). When increasing quantities of $ACTH_{1-39}$ were combined with a fixed quantity of $[Trp(Nps)^9]ACTH_{1-39}$, the response was increased from βB_{max} to αB_{max} (right panel, Fig. 1). The observed responses fit the mathematical model of Ariëns and Simonis (5) for combination of an agonist and a partial agonist

$$\frac{B^{a+p}}{B_{max}} = \frac{\alpha A}{A + (1 + P/P_{50})A_{50}} + \frac{\beta P}{P + (1 + A/A_{50})P_{50}} \qquad [1]$$

where B^{a+p} is the response observed with the combination of agonist and partial agonist. Estimates of apparent dissociation constants A_{50} and P_{50} for $ACTH_{1-39}$ and $[Trp(Nps)^9]ACTH_{1-39}$ acting in combination were 16.7 fmoles and 2640 fmoles, respectively; β, 0.59. The lines of Fig. 1 represent the values of B^{a+p}/B_{max} calculated

from Equation 1; the fit of the observed values (the points) to the lines is excellent. From the evidence presented we believe that [Trp(Nps)^9]$ACTH_{1-39}$ is a partial agonist. The similarity of the constants determined from $ACTH_{1-39}$ and [Trp(Nps)^9]$ACTH_{1-39}$ acting alone and in combination suggest that $ACTH_{1-39}$ and its derivative compete reversibly for the same receptor site involved in steroidogenesis (7).

$ACTH_{1-24}$ induces the same B_{max} as $ACTH_{1-39}$ in the isolated cell system. In other words, the fragment, $ACTH_{1-24}$, has the same intrinsic activity as the natural $ACTH_{1-39}$ ($\alpha = 1.0$). A preparation of [Trp(Nps)^9]$ACTH_{1-24}$ exhibited a β value of 0.71 when compared to $ACTH_{1-24}$. The Nps derivative was twice chromatographed on paper (see Materials and Methods) and again assayed. The chromatographed derivative exhibited the same β value as the starting material.

Summary. The o-nitrophenyl sulfenyl derivatives of $ACTH_{1-39}$ and $ACTH_{1-24}$ are partial agonists. They stimulate corticosterone production when added to suspensions of isolated adrenal cells. Maximum rate of steroidogenesis induced by these derivatives is less than that induced by the agonists $ACTH_{1-39}$ and $ACTH_{1-24}$. In appropriate doses the derivatives antagonize the steroidogenic action of the agonists. These observations emphasize the importance of tryptophan at position 9 of the ACTH molecule in the process involved in excitation of receptor.

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