102. Hormone: Receptor Interactions. Biological Activities of [Phenylalanine^2, norvaline^4]-adrenocorticotropin-(1-24)--tetrakosipeptide and its 4,5-Dehydro-4,5-ditritio-norvaline^4 Analogue in Isolated Rat Lipocytes and Adrenal Cortex Cells: Lipolysis, Corticosterone and *cyclic* Adenosine-3′,5′--monophosphate Production

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Zusammenfassung. [Phenylalanin^2, norvalin^4]-adrenocorticotropin-(1-24)-tetrakosipeptid und sein 4,5-dehydro-4,5-ditritio-Derivat sind in isolierten Ratten-Lipocyten und -Nebennierenrindenzellen biologisch gleich aktiv. In Bezug auf Lipolyse, Corticosteron- und *cyclo*-AMP-Produktion zeigen sie gleiche intrinsische Aktivität wie das Adrenocorticotropin-(1-24)-tetrakosipeptid mit der natürlichen Aminosäurensequenz, müssen aber zur Erzielung desselben Effektes in ca. 8–12mal höheren Dosen angewandt werden.

Using 'classical' methods of peptide synthesis and fragment condensation according to the general scheme of *Kappeler* & *Schwyzer* [1], *Karlaganis* & *Schwyzer* [2] have prepared the chemically pure ACTH analogues [phenylalanine^2, norvaline^4]-adrenocorticotropin-(1-24)-tetrakosipeptide, **1**, and [phenylalanine^2, (4,5-dehydro-4,5-di-

H · Ser-xxx-Ser-yyy-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-
1 5 10

-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro · OH
15 20 24

	xxx	yyy
1	Phe	Nva
2	Phe	Nva (t_2) = —NHCH(CH_2—CHT—CH_2T)CO—
3	Tyr	Met
4	Phe	Agl = —NHCH(CH_2—CH=CH_2)CO—

tritio)-norvaline[4]]-adrenocorticotropin-(1-24)-tetrakosipeptide, **2**[1]). The polypeptides **1** and **2** were obtained from fully protected [phenylalanine[2],L-allylglycine[4]]-adrenocorticotropin-(1-24)-tetrakosipeptide by catalytic hydrogenation and tritiation, respectively. Compound **2** contains > 90% of the tritium label in the norvaline[4] side chain and displays a specific activity of 7.42 Ci/mmol.

Lipolytic activity (= velocity of glycerol formation) was determined with isolated rat lipocytes [3] according to methods previously described in more detail by *Lang & Schwyzer* [4]. The results were based on a comparison with adrenocorticotropin-(1-24)-tetrakosipeptide, **3** (Synacthen®) [1], and are displayed in Fig. 1 as 'log dose: response

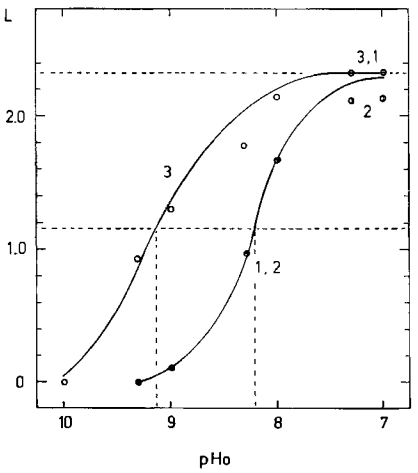

Fig. 1. *log Dose response plots for lipolysis in aliquots of a single suspension of rat epididymal lipocytes in response to* $ACTH_{1-24}$, **3** (○), [*Phe*[2], *Nva*[4]]-$ACTH_{1-24}$, **1** (⊖), *and* [*Phe*[2], *Nva*(t_2)[4]]-$ACTH_{1-24}$, **2** (⊙). Abscissa: pHo = negative logarithm of hormone concentration in mol/l; ordinate: velocity of lipolysis (L) in ng of glycerol produced per mg dry cell weight per 30 min. Means from 4 different assays and possible S-shaped curves for the log dose response relationship; *cf.* Table.

curves'. As expected, **1** and **2** exhibited practically identical characteristics, and curves generally quite similar to that of **3**. The *intrinsic activities* (maximum velocities) are practically the same for all three compounds; the analogues are, however, about 10 times less *potent* than the compound with the natural sequence, *cf.* Table.

In isolated rat adrenal cortex cells, prepared according to *Sayers, Swallow & Giordano* [5], the velocities of corticosterone (B) and *c*AMP production were determined following the procedures published in detail by *Sayers et al.* [6] [7]. A comparison was made between $ACTH_{1-24}$, **3**, and [Phe[2],Nva[4]]-$ACTH_{1-24}$, **1**. Although the compound was not tested, we assume that labelled **2** would behave like **1**. As shown in Fig. 2, the general shapes of the 'log dose: response curves' obtained for **1** and **3** are very similar. The *intrinsic activities* for the two compounds are the same for corti-

[1]) *Abbreviations:* ACTH = adrenocorticotropic hormone; [Phe[2], Nva[4]]-$ACTH_{1-24}$ = **1**; [Phe[2], Nva (t_2)[4]]-$ACTH_{1-24}$ = **2**; $ACTH_{1-24}$ = **3**; *c*AMP = *cyclic* adenosine-3′,5′-monophosphate; B = corticosterone.

Intrinsic Activities and Potencies of [Phe², Nva⁴]-ACTH$_{1-24}$, **1**, *[Phe², Nva(t_2)⁴]-ACTH$_{1-24}$*, **2**, *and ACTH$_{1-24}$*, **3**, *in Isolated Rat Lipocytes and Adrenal Cortex Cells*

	1	**2**	**3**
Maximum rate of glycerol production ± one standard error (L_{max} ± S.E.) [a]	2.32 ± 0.1	2.29 ± 0.1	2.12 ± 0.2
‚Lipolytic' potency in mol/l (= dose required for $^1/_2$ L_{max})	6.7 ± 0.5 $\times 10^{-9}$	6.7 ± 0.5 $\times 10^{-9}$	7.5 ± 1 $\times 10^{-10}$
Maximum rate of corticosterone production ± one standard error (B_{max} ± S.E.) [b]	1.39 ± 0.02	–	1.40 ± 0.04
‚Steroidogenic' potency in mol/l (= dose for $^1/_2$ B_{max})	2.05 ± 0,15 $\times 10^{-10}$	–	2.7 ± 0.4 $\times 10^{-11}$
Maximum rate of *c*AMP (8-^{14}C) production ± one standard error ($cAMP_{max}$ ± S.E.) [c]	583 ± 17	–	605 ± 25
‚Cyclase' potency in mol/l (= dose for $^1/_2$ $cAMP_{max}$)	6.36 ± 0.93 $\times 10^{-9}$	–	5.15 ± 0.12 $\times 10^{-10}$

a) 100% $L_{max} \equiv 2.32$ ng of glycerol per mg dry cell weight [4] per 30 min. Means and S.E. from 4 assays.

b) B_{max} in μg per cell suspension aliquot [5] [6] per 60 min. Regression coeff. ± S.E.

c) *c*AMP$_{max}$ in cpm of *c*AMP (8-^{14}C) [7] per cell suspension aliquot per 60 min. Regression coeff. ± S.E.

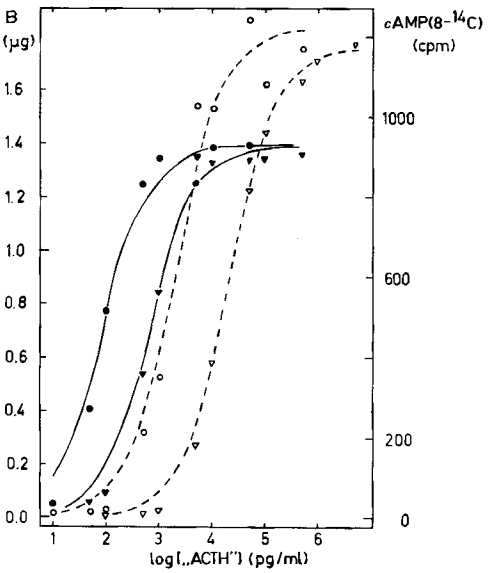

Fig. 2. *log Dose response plots for cAMP(8-^{14}C)* (– – –) *and corticosterone (B)* (——) *production by aliquots of a single suspension of isolated rat adrenal cortex cells in response to ACTH$_{1-24}$*, **3** (○ *or* ●), *and [Phe², Nva⁴]-ACTH$_{1-24}$*, **1** (▽ *or* ▼). The points are the means of analyses on two aliquots of cell suspension; the curves represent nonlinear least square fits. Abscissa: log hormone dose in pg/ml; left ordinate; µg corticosterone (B) produced in 60 min.; right ordinate: counts per min. of *c*AMP(8-^{14}C) produced from ATP(8-^{14}C) in 60 min.; *cf* Table.

corticosterone as well as for *c*AMP production; however, the *potency* of the analogue, **1**, is, compared to **3**, reduced by a factor of 8 in the first, and of 12 in the second instance, *cf.* Table.

Analogues of ACTH with phenylalanine replacing tyrosine in position 2 and norvaline replacing methionine in position 4 have been described previously. [Phe^2]-ACTH$_{1-23}$-amide, **4**, is reported to display about 50% of the potency of ACTH [8], [Nva^4]-ACTH$_{1-25}$-amide has a 'biological activity... of the same order of magnitude as that of natural ACTH' [9], as determined by ascorbic acid depletion and other, unspecified pharmacological tests, respectively.

We can tentatively conclude that our two analogues, **1** and **2**, react with essentially the same receptor molecule populations in the two cell preparations as the 'natural' tetrakosipeptide, **3**, but that their *'affinities'* (pharmacological theory, *cf.* [10]) for the discriminator moieties are, in the mean, 10 times smaller. This is borne out by recent observations of reversible association of **2** with isolated fat cells [11] [12].

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