VARIABILITY OF THE DURATION OF INHIBITION OF GROWTH HORMONE RELEASE BY N^{α}-ACYLATED-DES-[Ala^1-Gly^2]-H_2SOMATOSTATIN ANALOGS.

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SUMMARY: While showing consistent and easily reproducible results as observed in vitro or in acute in vivo systems, several N^{α}-acylated-des-[Ala^1-Gly^2]-dihydrosomatostatin analogs exhibit variable protracted inhibition of the growth hormone release induced in rats by pentobarbital. These results may reflect variable central nervous system responses to pentobarbital as well as to the somatostatin analogs.

INTRODUCTION

Somatostatin (SRIF) and dihydrosomatostatin (H_2SRIF) acutely inhibit growth hormone (GH) release induced in rats by pentobarbital (PB) (1). A recent communication from this laboratory reported the biological potency of a series of N^{α}-acylated-des-[Ala^1-Gly^2]-dihydrosomatostatin analogs (acyl-Cys^3-H_2SRIF) when tested in vivo and in vitro with SRIF as a reference standard in the acute tests described earlier (2). In the same note it was reported that these compounds exhibited extended duration of action (up to 72 hrs) on inhibition of growth hormone release induced by pentobarbital in rats (2). While we have repeatedly confirmed the reported specific activities (u/mg) of these various analogs in the acute tests in vivo and in vitro on inhibition of GH-release, we have now found that the reported protracted biological effects are inconsistent (3); a working hypothesis is offered as a possible approach to explain these inconsistencies.

METHOD AND MATERIALS

Rat Bioassay.

Male Sprague-Dawley rats weighing 100g. were used in all experiments. Animals were fed tap water and Purina Rat Chow ad lib and housed in temperature and humidity controlled quarters with 14 hours of light and 10 hours of dark (light 0700-2100). All experiments were performed between 1400 and 1600 on animals received from the supplier at least five days previously. At the time intervals after administration of H_2SRIF analogs (subcutaneously, 100 µg/0.5 ml saline), as indicated below, animals were ether anesthetized and saline or

pentobarbital (2 mg/100g BW) was given via the external jugular vein. Ten minutes after these IV injections the animals were decapitated and trunk blood collected for radioimmunoassay of growth hormone.

Growth Hormone Radioimmunoassay.

Plasma GH determinations were made utilizing the following reagents: NIAMDD rat GH standard (GH-RP-1); NIAMDD monkey anti-rat GH (GH-serum-3); highly purified rat GH for iodination obtained from Dr. Stanley Ellis, Ames Research Center, Moffett Field, California.

Peptides.

The various N^{α}-acylated-des-[Ala^1-Gly^2]-dihydrosomatostatin analogs were synthesized in this laboratory as previously described (2). In view of the possible instability of those derivatives toward air oxidation, new preparations were made and compared to the original ones (often more than one year old) which had been kept as white lyophilized powders in closed vials at 0°C. Peptide content by amino acid analyses (90-100%), sulfhydryl content according to Habeeb (4) (50-80%) and the integrity of the tryptophan residue when calculated on the basis of an extinction coefficient of 6.200 at 280 nm (80-90%) showed both new and old batches to be comparable. It is evident that some material has polymerized but it is of interest to note that in acute studies, those analogs were fully potent. We conclude that, reduction and oxidation of those materials in the biological systems used take place very rapidly.

Statistical Analyses.

Following analysis of variance, differences between treatment means were analyzed by the multiple range tests of Dunnett or Duncan. All calculations were carried out by the UCLA IBM 360 Model 95 computer with the program EXBIOL. Since there was in most experiments considerable heterogeneity of the variances of the means between various experimental groups, all statistical analyses as above were performed on $\log_{10}$ transforms of the original values (GH ng/ml plasma shown in Table 1).

RESULTS AND DISCUSSION

Table 1 shows the effects of various des-[Ala^1-Gly^2]-acyl-Cys^3-H_2SRIF analogs (100 µg/100g BW) given 24 or 48 hours prior to PB. It is evident from these data that variable inhibition of pentobarbital induced GH-release is seen with these peptides. Significant inhibition of GH-release induced by

TABLE 1. EFFECTS OF N^{α}-ACYL-CYS^{3}-DIHYDROSOMATOSTATIN ANALOGS ON GH RELEASE INDUCED BY PENTOBARBITAL (PB) [1]

Experimental Protocol No.	Acyl-Cys^{3}-H_2SRIF Analog [2]	t [3]	PB + Saline [4]	PB + Analog	P
15500	Bzl-Cys^{3}-H_2SRIF	24	388 ± 85	218 ± 61	>.05
15520	Bzl-Cys^{3}-H_2SRIF	24	1014 ± 256	1160 ± 457	>.05
	Bzl-Cys^{3}-H_2SRIF	48	483 ± 164	186 ± 78	>.05
15591	Bzl-Cys^{3}-H_2SRIF	24	1096 ± 199	345 ± 160	<.05
	Acr-Cys^{3}-H_2SRIF	24	1096 ± 199	340 ± 143	<.05
	Pyv-Cys^{3}-H_2SRIF	24	1096 ± 196	277 ± 92	<.01
15599	Bzl-Cys^{3}-H_2SRIF	24	713 ± 234	878 ± 297	>.05
	Acr-Cys^{3}-H_2SRIF	24	713 ± 234	778 ± 153	>.05
	Hex-Cys^{3}-H_2SRIF	24	713 ± 234	630 ± 239	>.05
	Pyv-Cys^{3}-H_2SRIF	24	713 ± 234	797 ± 294	>.05
	Bzl-Cys^{3}-H_2SRIF	48	713 ± 234	573 ± 144	>.05
	Acr-Cys^{3}-H_2SRIF	48	713 ± 234	681 ± 126	>.05
	Hex-Cys^{3}-H_2SRIF	48	713 ± 234	1496 ± 370	>.05
	Pyv-Cys^{3}-H_2SRIF	48	713 ± 234	271 ± 60	<.05
15638	Bzl-Cys^{3}-H_2SRIF	24	628 ± 167	179 ± 89	<.05
	Ac-Cys^{3}-H_2SRIF	24	628 ± 167	167 ± 74	<.05
15725	Ac-Cys^{3}-H_2SRIF	24	344 ± 164	358 ± 118	>.05

[1] PB administered IV at 2 mg/100g BW.

[2] Acyl groups are as follows: Benzoyl (Bzl), Acrylyl (Acr), Pyvalyl (Pyv), Hexanoyl (Hex), Acetyl (Ac).

[3] Time in hours of administration of peptide prior to PB. All peptides were given subcutaneously, 100 µg/100g BW/.5 cc. in saline.

[4] Absolute control values for GH were always <50 ng/ml. Each group represents the mean ± SEM of six animals.

pentobarbital is observed in several experiments but not in any predictable fashion.

Recent observations in several laboratories including our own, offer a working hypothesis to further investigate these results: 1) There is good evidence that pentobarbital acts on some site(s) in the central nervous system to induce release of growth hormone (5,6); 2) Two hypothalamic hypophysio-

tropic peptides, thyrotropin releasing factor (TRF) and somatostatin have been shown to act on the central nervous system independently of their effects at the pituitary level: somatostatin potentiates anesthesia time due to pentobarbital and decreases its LD_{50}; also it shortens the duration of strychnine-induced seizures and increases the strychnine LD_{50} (7,8); TRF has opposite actions on the pharmacological effects of the same drugs; 3) The release of growth hormone induced by pentobarbital is inhibited by TRF, only in in vivo system - no effects of TRF on GH-release by cell cultures of normal rat pituitary glands (6). Thus, with knowledge of multiple central nervous systems sites for control of GH secretion and with the evidence of extra pituitary CNS effects of somatostatin it is possible that the end effect observed here in this experimental protocol (release of GH by pentobarbital as affected by somatostatin analogs in a chronic treatment - 24 to 48 hrs) may be more related to effects of the various substances on central nervous system of the test animals than on the pituitary alone. In favor of this interpretation is also the earlier series of observations showing the various acyl-Cys^3 analogs of dihydrosomatostatin to have greater inhibitory activity (on the release of GH induced by pentobarbital) when studied in vivo in acute tests than when similarly studied in vitro (See Table 2, in 2), a point observed earlier and for which we had no obvious explanation. Indeed, the observed potency to inhibit secretion of GH by anyone of these somatostatin analogs when tested in vivo in the rat would be the summation of its hypophysiotropic activity and of its CNS-neurotropic activity.

The multiplicity of the factors possibly involved in the CNS mediation of GH-release in similarly prepared laboratory animals as reported here will make it imperative to study a large number of multiple variables including systematic pretreatment times other than the 24 and 48 hrs. timings studied here. The data reported here further suggests that great care be taken in interpretation of results obtained on inhibition of GH release in vivo in the rat. If TRF is as active as somatostatin in this test (6) while not active in vitro on pituitary cells to inhibit release of GH, the specificity (i.e. action directly at pituitary level) of results observed in the in vivo method must be questioned until confirmed in a valid in vitro system.

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