PURIFICATION OF A PROLACTIN INHIBITING HORMONE AND THE REVEALING OF HORMONE D-GHIH WHICH INHIBITS THE RELEASE OF GROWTH HORMONE

by

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SUMMARY

A prolactin inhibiting factor (hormone, PIF, PIH) has been purified from porcine hypothalami. Inhibition, in vitro, of prolactin release was observed at a dosage of approx. 10-100 ng, estimated on the basis of uv absorption. The same fraction at the same dosage also inhibited the release of the growth hormone. The entities behaving like PIH and GHIH were separated by high pressure liquid chromatography. The PIH activity was inactivated by pronase. The entity showing GHIH-activity and synthetic somatostatin appear to be different. Since hypothalamic hormones frequently show multiple activities, this entity is provisionally named hormone D-GHIH until it is chemically and biologically characterized. The D-GHIH designation merely defines the assay guiding isolation.

INTRODUCTION

The existence of a prolactin inhibiting factor (PIF) was first independently and concomitantly described by Talwalker, Ratner and Meites[1] and by Pasteels.[2] Later, Schally, et al.[3] reported that the release of prolactin (PRL) by the rat pituitary was inhibited by bovine, ovine and porcine hypothalamic extracts, and observed that PIF was not species specific. PIF was partially purified by Dhariwal, et al.[4], and was found difficult to separate from the luteinizing hormone releasing hormone by Arimura, et al.[5] Recently, Dular, et al.[6] described fractions from bovine posterior pituitary-stalk-median eminence which inhibited, in vitro, release of PRL and to a lesser extent that of the growth hormone (GH) and thyrotropin (TSH). More purified fractions only inhibited release of PRL. Krulich, et al.[7] and Stachura, et al.[8] reported on the inhibition of the release of GH. Brazeau, et al.[9], Burgus, et al.[10], and Rivier, et al.[11]

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reported on the isolation, from ovine hypothalami, the structure and the synthesis of somatostatin (SRIF), which was found to inhibit the secretion of GH.

We have purified (PIH, PIF), and observed that PIH-containing fractions frequently inhibited also the release of GH. It became important to differentiate the entity inhibiting the release of GH from somatostatin and from PIH. We describe this experience.

METHODS AND BIOASSAYS

Batches of 5,000 lyophilized porcine hypothalamic fragments were homogenized and extracted with methanol/acetic acid. The extract was evaporated, dissolved in water, defatted with methylene chloride and extracted with butanol. The aqueous phase was evaporated. The residue was applied to a Bio-Gel P-2 column (1800 Daltons). After rechromatography on the same column, further purification was accomplished with two different partition chromatography systems on Sephadex G-25: System A, 0.1% acetic acid-butanol-pyridine (11:5:3); System B, butanol-acetic acid-water (4:1:5). Then DEAE-Sephadex with an acetic acid gradient was used. Next, we used the equipment for high pressure liquid chromatography (hplc) with Poragel-PT and Phenyl-Porasil/Corasil as packing materials of Waters Associates, Inc. Active PIH fractions were incubated with pronase[12] (Sigma Chemical Co.) for 34 hr. at 37°C in 50 mM sodium phosphate, pH 7.4. Enzyme and buffer controls were also bioassayed.

For the in vitro assays, pituitaries were obtained from 20-day old female rats of the Sprague-Dawley strain. Two pituitaries for each assay were incubated at 37°C in 1 ml of lactated Ringer's Solution (Travenol Laboratories) in 10-ml Teflon beakers in a Dubnoff Shaker. After pre-incubation for 1 hour, the medium was discarded and replaced. After a second hour of pre-incubation, the medium was removed for assay (P_2) and replaced. The samples were added after the two-hour pre-incubation period; the total time was 3 hours (I_3). The radioimmunoassay method of Daane and Parlow[13] was used for measuring PRL and GH. The reagents for the PRL and GH assays were provided by NIAMD, NIH.

RESULTS AND DISCUSSION

Table 1 shows bioassay results on hormonal activities for inhibition of release of PRL and GH for fractions at different purification steps. These results are representative of a large number of similar purifications by the same chromatographic methods. Table 2 shows the time response of prolactin release to a PIH-active fraction, compared with the effect of saline, LHRH and an inactive fraction. Table 3 shows the effect of dopamine and epinephrine on PRL release in our assay system. The effect of somatostatin on both PRL and GH levels is shown in Table 4.

On partition chromatography with System A, the GIH-activity was eluted with an R_f of about 0.15. In contrast, it has been reported that the cyclic soma-

Table 1. DATA ON HORMONAL ACTIVITIES FOR INHIBITION OF RELEASE OF PRL AND GH

Stage	Fraction No.	Dose µg	ng PRL/ml P_2	ng PRL/ml I_3	ng GH/ml P_2	ng GH/ml I_3
1st P-2	60- 90	150	37	35	344	510
"	91-110	250	58	28	545	647
"	111-130	1000	36	25	335	580
"	131-160	15000	35	7	258	235
"	161-190	4000	60	10	763	533
"	191-310	400	26	5	219	203
"	131-150	25000	50	41	270	523
"	151-175	7000	109	42	1010	130
"	176-200	800	108	7	345	<20
"	201-300	150	89	45	340	600
"	301-600	150	90	69	575	680
2nd P-2	88-140	5000	49	40	565	1206
"	141-160	7000	45	25	830	251
"	161-180	3000	61	18	926	134
"	181-190	1500	40	1	372	113
"	191-200	200	45	15	593	244
System A-PC	56- 85	500	51	42	925	559
"	86-100	250	45	22	252	236
"	101-125	250	40	1	516	106
System B-PC	51- 70	300	52	16	798	651
"	71- 90	600	28	4	1105	252
"	91-110	150	16	11	803	681
System B-PC	51- 55	50	46	37	510	280
"	56- 80	200	48	25	540	180
"	81- 85	15	61	21	1090	320
"	86- 95	15	53	27	300	200
DEAE-S	0.2M HOAc	30	62	34	712	1069
"	1 M HOAc-1	25	78	24	604	100
"	1 M HOAc-2	50	52	42	1606	1426
DEAE-S	1 M HOAc-1	50	94	67	1380	1943
"	1 M HOAc-2	50	32	7	1220	376
"	1 M HOAc-3	50	30	28	188	362
HPLC-PT	80% MeOH-1	0.01-0.1	59	13	517	64
"	80% MeOH-2	<0.01	67	51	1157	557
HPLC-Phenyl	H_2O-1	0.5-0.25	23	19	350	0
"	H_2O-2	15	32	10	213	200

tostatin had an R_f of 0.4.[10] On a column of carboxymethyl-cellulose, the GIH-activity was not retained with 2 mM NH_4OAc (pH 6.7), while somatostatin reportedly is retained.[10] Synthetic somatostatin was strongly retained by hplc on Poragel-PT while the GIH-activity was not retained.

In most of the preparative purifications according to the data in Table 1, the activities of PIH and GIH closely followed each other but were separated by hplc (Phenyl). A partial separation also was obtained on Sephadex G-10. Treatment of PIH fractions with pronase[12] resulted in inactivation.

Table 2. IN VITRO EFFECT OF PARTIALLY PURIFIED PORCINE PIH ON RELEASE OF PRL FROM PITUITARIES OF 20 DAY OLD FEMALE RATS

Additions to incubation medium	ng PRL/ml medium				
	P_1	P_2	I_3	I_4	I_5
Saline	426±120	343±82	279±48	256±71	161±31
LHRH 0.3 ng	583± 16	462±92	352±59	324±49	323±60
Inactive PIH Fraction	484± 41	462±68	372±26	280±36	229±27
Active PIH Fraction	517± 91	408±46	110±57	57±24	26±10

Mean of 3. p value (P_2 vs I_3 - I_5) = <0.02 for PIH; others ns.

Table 3. IN VITRO RELEASE OF PRL FROM PITUITARIES OF 20 DAY OLD FEMALE RATS

Additions to incubation medium				ng PRL/ml medium					
		I_3-I_4	I_5-I_6	P_1	P_2	I_3	I_4	I_5	I_6
-	-	-	-	130	95	93	95	125	63
-	-	-	-	133	108	105	100	85	63
-	-	-	-	170	125	120	120	115	65
Dopamine	ng/ml	10	40	315	240	82	88	61	<5
Dopamine	ng/ml	320	1280	158	95	29	16	18	<5
Epinephrine	ng/ml	20	20	288	208	138	111	143	112
Epinephrine	ng/ml	1000	1000	348	378	215	130	75	60

Table 4. IN VITRO EFFECT OF SOMATOSTATIN ON RELEASE OF PRL AND GH FROM PITUITARIES OF 20-DAY OLD FEMALE RATS

Synthetic Somatostatin	ng PRL/ml		ng GH/ml	
	P_2	I_3	P_2	I_3
1 μg/ml	40	25	285	145
10 μg/ml	44	30	395	330
50 μg/ml	55	31	325	400

The purified PIH corresponding to fraction hplc-PT in Table 1 gave an 80% decrease in the release of prolactin at an estimated dose level of 10-100 ng which was based upon the uv absorption at 254 nm of the whole fraction, the uv absorption of fractions of known weight and of synthetic peptides. This same

fraction at the same dosage range gave about a 90% decrease in release of GH. In our assay, somatostatin gave significantly less decrease of GH even at 1-50 μg-dose-levels (Table 4).

It is now well recognized that hypothalamic peptide hormones can have more than one hormonal activity. pGlu-His-Pro-NH_2 releases both TSH and PRL. The synthetic luteinizing hormone releasing hormone releases both LH and FSH. Somatostatin inhibits the rise of GH levels after insulin-induced hypoglycemia, exercise, levodopa and arginine and lowers GH levels in acromegalic patients. Also, it inhibits the rise of TSH induced by TRH or the insulin and glucagon rise after arginine.[14,15]

Until the entity we have highly purified and which inhibits the release of GH is chemically and biologically characterized, it seems appropriate to designate it as hormone D-GHIH. The abbreviation GHIH has been linked to the letter D just to indicate the biological activity which is guiding the isolation of the substance.

The inhibition of release of prolactin by catecholamines is under current extensive study by many investigators. Shaar and Clemens[16] considered that a catecholamine may be a PIF. Schally, et al.[17] observed at least four types of fractions with characteristics different from those of catecholamines which inhibited the release of prolactin. The results of Samli and McCloud[18] were consistent with dopamine being PIF. Lawson and Gala[19] observed that the control of prolactin secretion is complex. We have examined the effect of the two catecholamines, dopamine and epinephrine, on prolactin release, and found that dopamine was active in ng-doses in our system (Table 3). We were, however, able to separate dopamine from PIH by ion exchange chromatography. While both dopamine and norepinephrine were eluted with 2-30 mM NH_4OAc on DEAE-Sephadex, PIH was strongly retained, and eluted with 1 M HOAc.

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