Interference of Immunoglobulins in the Radioimmunoassay of Human β-Endorphin

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Summary: The interference of immunoglobulins in the radioimmunoassay (RIA) of human β-endorphin was investigated. Human IgM showed no cross-reactivity. Human IgA showed a weak cross-reaction, but the dilution curve of IgA did not show parallelism with the standard curve of β-endorphin, thus indicating its antigenic difference. The dilution curves of human IgG showed 0.18% displacement with respect to the human β-endorphin standard curve, with good parallelism. Moreover, five patients with multiple myeloma of the IgG type showed falsely elevated β-endorphin levels. We investigated the possibility that certain IgGs may be responsible for the displacement of $[^{125}I]$β-endorphin in the β-endorphin kit. The apparent β-endorphin level of plasma from multiple myeloma patients was markedly decreased after affinity chromatography of the serum on protein A-Sepharose.

In another 3 patients with multiple myeloma, we examined IgG interference by measuring the β-endorphin levels in their lyophilized IgG diluted with saline. The results demonstrated high values of 20.2, 25.5 and 21.2 pmol/l respectively, also showing good parallelism. These immunological parallels to human β-endorphin verify that a part of the amino acid sequence of human IgG is similar to that of human β-endorphin.

Consequently, in the measurement of β-endorphin with polyclonal antibody, the results may sometimes be spuriously high due to cross-reaction with IgG, e. g. in patients with IgG myeloma. To avoid IgG interference, a specific monoclonal antibody to synthetic β-endorphin should be used rather than polyclonal antibodies.

Introduction

Many reports on β-endorphins have been published during the past decade (1 — 9). Clinical studies indicate that the intravenous administration of synthetic β-endorphin exerts beneficial biological effects on patients with cancer pain (10), in narcotic abstinence syndrome (10), in schizophrenic behaviour, and depression (11). An interesting finding has been reported by *Julliard* et al. (12), who discovered an apparent structural similarity between a high molecular weight immunoreactive β-endorphin and a fragment of immunoglobulin G (IgG$_1$) in extracts from the human placenta. Surprisingly, considerable structural similarities between corticotropin and a fragment of IgG were also revealed. These findings stimulated our interest in the connection between the structures of hormones and immunoglobulins and gave rise to several interesting questions.

1) Does β-endorphin radioimmunoassay show immunoreactivity, not only with human IgG, but also with IgA and IgM?

2) Does circulating IgG interfere with the human β-endorphin radioimmunoassay?

3) Does human IgG show immunoreactivity in other human pituitary hormone radioimmunoassays?

No information concerning these questions has hitherto been reported. We therefore investigated the influence of human IgG on the radioimmunoassay for human pituitary hormones, including β-endorphin in order to elucidate possible existing homologies in the amino acid sequences of these physiologically unrelated substances.

Normal subjects and patients

Normal β-endorphin values were determined in 82 normal healthy volunteers (43 males; 49 females; aged 18−65) whose blood was collected between 0800 and 0900. The blood samples (5−10 ml) were collected in plastic tubes by venipuncture containing 2 g/l EDTA; centrifuged within 15 min, the plasma separated and immediately frozen at −20 °C. Six of the volunteers were given metyrapone, 2.0 g orally at 9 a.m., with blood samples drawn immediately beforehand and after 6 h and 8 h. From the volunteer group blood samples for β-endorphin, corticotropin and cortisol were obtained from 6 subjects at 11 p.m., followed by the administration of 1.0 mg of dexamethasone orally. At 6 a.m. and 8 a.m. further blood samples were obtained. Six more normal subjects underwent an insulin tolerance test (monocomponent insulin 0.1 U/kg). During this test blood samples were taken via an intravenous cannula at −30, −15, 0, 15, 30, 60, 90, and 120 min and assayed for glucose, corticotropin, β-endorphin, cortisol and insulin.

Five patients with pituitary corticotropin-dependent *Cushing*'s disease who had been bilaterally adrenalectomized and five patients with *Addison*'s disease receiving corticosteroid replacement therapy were studied. Replacement therapy was omitted on the morning of the study; venous blood samples were taken at 0900 h and thereafter at 30 min intervals for 3 h, and were assayed for corticotropin, β-endorphin and cortisol. Five patients with multiple myeloma of the IgG type (3 Lambda, 2 Kappa) had plasma taken for β-endorphin assay. These samples were assayed with and without affinity chromatography on staphylococcal protein A-Sepharose.

Materials and Methods

Concentrations of immunoreactive β-endorphin were determined by means of a commercial radioimmunoassay kit developed by the New England Nuclear Company, Boston, MA, USA, with antiserum from rabbits prepared against synthetic human β-endorphin (synthesized by the Peptide Division of the Beckman Company, FRG). Synthetic β-endorphin was labelled

with Na^{125}I using chloramine T. The specific activity of [^{125}I]β-endorphin ranged from 56.1 to 83.6 TBq/mmol. The assay was performed according to the following protocol. Briefly, 0.1 ml specimen was used. Synthetic human β-endorphin served as a standard. The antiserum to β-endorphin was used at a final dilution of 1 : 8000. Separation of the bound, from free antigen, was achieved by adsorption onto activated charcoal. The specificity of the β-endorphin antiserum was 50% for β-lipotropic hormone (β-lipotropin), less than 0.01% for α-endorphin and α-melanocyte stimulating hormone (α-melanotropin), and less than 0.004% for leu-enkephalin and met-enkephalin. The minimal detectable dose was 5.0 pmol/l. The intra- and interassay variation were 5.6% and 12% respectively. To ensure the accuracy of our RIA in the determination of human plasma β-endorphin levels, we performed dexamethasone supression tests, insulin hypoglycaemia tests and metyrapone tests on normal healthy subjects; the results were in agreement with previously reported values for β-endorphin in these tests. In our laboratory the β-endorphin levels ranged from 5−30 pmol/l.

Highly purified human IgG was obtained from Cappel Labs West Chester, PA, USA, (lot no. 29153), Kabi Co. (lot no. 58803), Sweden, and Miles Labs, Indiana, USA (lot no. 8376); human IgA from Miles Labs (lot no. 21277) and human IgM from Miles Labs (lot no. 9675). Human immunoglobulins (IgG, IgA and IgM) were measured by laser nephelometry (Hoechst Co. FRG). The M-protein types and light chain types were identified by Beckman Paragon Immunofixation Electrophoresis (Beckman, Brea, CA, USA). For affinity chromatography of the samples on Protein A-Sepharose, we used the Immunopure IgG Purification Kit (Pierce Chemical Co., USA) according to the manufacturer's instructions.

Results

Plasma β-endorphin concentrations

Table 1 shows the circulating plasma β-endorphin levels in 82 healthy normal subjects, 5 bilaterally adrenalectomized patients with *Cushing*'s disease and 5 patients with *Addison*'s disease. During the dexamethasone suppression tests the plasma β-endorphin levels fell in each subject and rose during the insulin-induced hypoglycaemia and metyrapone tests. All the patients with *Cushing*'s disease and *Addison*'s disease had elevated plasma β-endorphin and corticotropin concentrations.

Tab. 1. Concentrations (mean ± SD) of plasma β-endorphin (pmol/l) in normal subjects, patients with bilaterally adrenalectomized *Cushing*'s disease and *Addison*'s disease.
* P < 0.01, ** P < 0.001

Subjects	n	Basal level	Post metyrapone	Post dexamethasone	Post insulin hypoglycaemia
Normal males	43	7.9 ± 0.9			
Normal females	49	7.7 ± 1.1			
Normal males	6	7.7 ± 0.8	26.1 ± 2.1* (a)	<5.0 ~ 6.1* (a)	
Normal males	6	7.8 ± 1.0			18.2 ± 3.2* (a)
Cushing's disease	5	68.2 ± 5.6** (b)			
Addison's disease	5	91.5 ± 11.2** (b)			

Statistical analysis: (a) Paired t test (basal level vs post drug administration)
(b) *Student* t test

Tab. 2. Plasma β-endorphin levels and related data in 5 patients with multiple myeloma

Pa-tient No.	Sex	Age (a)	Protein concentrations (g/l)						Immunoglobulin concentrations (mg/l)			Light chain type	β-Endorphin concentrations (pmol/l) without with	
			To-tal pro-tein	Al-bu-min	α1-	α2-	β-	γ-	IgG	IgM	IgA			
					globulins								purification by protein A-Sepharose	
1	♂	37	113	36.6	1.6	4.6	4.6	65.5	69100	460	680	Kappa	23.4	9.1
2	♀	40	86	39.8	2.6	5.2	3.9	34.6	36300	620	1500	Lambda	16.3	8.8
3	♀	44	101	33.8	6.6	11.6	8.1	40.9	49250	390	980	Lambda	18.1	8.2
4	♂	67	112	35.8	6.7	14.6	4.5	50.4	53200	420	720	Lambda	15.2	5.2
5	♂	70	102	34.1	1.8	4.4	4.5	57.2	61150	510	950	Kappa	19.4	5.1
Reference ranges			66–81	39–49	1.0–3.5	5.5–11.8	4.3–9.0	5.5–14.1	13000–17740	600–1480	1780–3550		*18.5 ± 1.4	7.3 ± 0.9

$$* \; p < 0.01$$

Table 2 shows the β-endorphin levels in patients with multiple myeloma of the IgG type. All the patients showed high plasma β-endorphin levels but within the normal range, but then showed a marked decline after affinity chromatography on staphylococcal protein A-Sepharose, which suggests IgG interference.

The effect of human IgG, A, M, on the β-endorphin radioimmunoassay

The dilution curve of human IgA and IgM did not show parallelism with the human β-endorphin standard curve, suggesting the IgA and IgM do not possess sequence homologies to β-endorphin. In contrast, commercial human IgG causes a significant displacement of labelled β-endorphin, with good parallelism (fig. 1); i.e. the dilution curves of IgG from Miles Labs, Cappel Labs and Kabi company showed 0.20%, 0.18% and 0.16% cross-reactivity, respectively, with the standard curve for β-endorphin, thus indicating
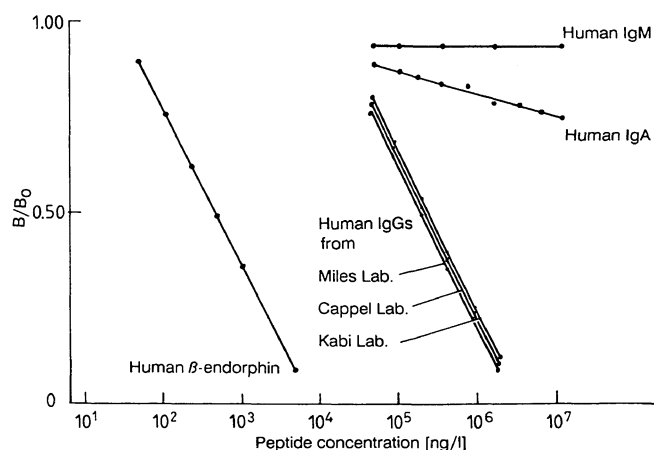
immunological identity. After affinity chromatography of the serum on staphylococcal protein A-Sepharose (13), no immunological cross-reactivity was observed.

The effect of IgG on human pituitary hormone radioimmunoassays

No immunological effect of IgG was found in the radioimmunoassays for lutropin, follitropin, thyrotropin, prolactin or somatotropin, indicating that there is no immunological similarity between the amino acid sequences of these hormones and that of IgG.

Discussion

The circulating human β-endorphin levels were checked by radioimmunoassay and were found to be 5 – 30 pmol/l. Moreover, the β-endorphin response in the dexamethasone suppression test, insulin hypoglycaemia test and metyrapone test showed results corresponding to those previously reported (14 – 17).

In 1980, *Julliard* et al. (12) discovered structual homologies between β-endorphin and IgG using a computer program. They synthesized a fragment of human IgG heavy chain 363 to 379 (VSLTCLVKGFYPSDIAV) by solid-phase techniques and reported that this fragment showed 0.18 percent displacement of the human β-endorphin tracer, with good parallelism. Our present study concurs with *Julliard*'s finding (12).

This result suggested that spuriously high β-endorphin levels may be obtained in patients with high γ-globulinaemia. Therefore a preliminary examination of three patients with IgG myeloma was carried out. Their lyophilized IgG, diluted with saline to a concentration of 177, 185, 162 g/l, indeed showed high

Fig. 1. Inhibition curves associated with various human immunoglobulins in the human β-endorphin radioimmunoassay.

β-endorphin values, i.e. 20.2, 25.5, 21.2 pmol/l, respectively, but showed negligible β-endorphin levels after the removal of IgG proteins by affinity chromatography on protein A-Sepharose. It is therefore concluded that the pseudo-β-endorphin activity of human plasma proteins is essentially due to the effect of IgG per se. To cross check the accuracy of these results we pre-added β-endorphin to the IgG solution from the same patients and carried out affinity chromatography. The subsequent recovery of β-endorphin was 95.6 ± 2.3% (Mean ± SE), thus demonstrating that there were no changes in concentration of β-endorphin in the IgG solution. This study clearly excludes the possibility that the β-endorphin is noncovalently linked to IgG. Moreover, in order to confirm our hypothesis we performed further experiments using the plasma from five other patients suffering from IgG myeloma. We studied the β-endorphin levels before and after staphylococcal affinity chromatography on protein A-Sepharose. As shown in table 2 the plasma β-endorphin levels were elevated before and markedly decreased after. These results clearly indicate IgG interference in the human β-endorphin radioimmunoassay.

Julliard et al. (12) reported that their studies had led to the unexpected recognition of several homologies in the amino acid sequences of two physiologically unrelated substances, such as pituitary hormone (β-endorphin and corticotropin) and the constant chain of an IgG. We therefore conducted a further study on the effect of IgG proteins on the radioimmunoassays of several pituitary hormones, in order to investigate the possibility of other unexpected relationships. In our studies of peptide hormones, no immunological similarities to IgG were found. However, the observation made by *Julliard* et al. (12) does seem important, and there may be other cases of substances arising from the same origin, which previously have been thought to be unrelated. Very recently a study by *Campino* et al. (18) described that immunoreactive human growth hormone, in a patient with acromegaly, is in fact IgG. Additional studies are expected.

Through our studies, it has become clear that to avoid IgG interference a specific monoclonal antibody which recognises the epitope of β-endorphin is preferable to using polyclonal antibodies raised in animals. The use of such a specific monoclonal antibody would avoid the spuriously high results for β-endorphin in patients with high IgG levels. Recently *Voellmy* et al. (19) reported a two-site immunoradiometric assay for human β-endorphin using a polyclonal rabbit anti-β-endorphin antibody and an ^{125}I-labeled monoclonal mouse antibody 3-E7, which binds to the N-terminal region of β-endorphin. If no IgG effect is demonstrated with this newly developed monoclonal antibody method, we can anticipate greater accuracy and reliability in the determination of β-endorphin concentrations.

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