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Respiratory Distress and Beta-Endorphin-Like Immunoreactivity in Humans

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Beta-endorphin-like immunoreactivity was determined in the plasma of twenty patients suffering from hypoxia of various etiologies and in twenty healthy adult volunteers who served as controls. Mean beta-endorphin-like immunoreactivity in the hypoxic patients was 53.2 ± 5.5 (SEM) pg/ml, as compared to the volunteer subjects in whom the mean level was 6.2 ± 1.9 pg/ml ($P < 0.01$). Significant negative correlations were present between both arterial pH ($r = -0.85$; $P < 0.01$) and arterial P_{O_2} ($r = -0.80$; $P < 0.01$) and beta-endorphin-like immunoreactivity. These findings seem to lend support to the hypothesis that hypoxia and acidosis represent stressful conditions which may stimulate the release of beta-endorphin in humans. (Key words: Acid-base equilibrium: acidosis. Hypoxia. Polypeptides: endorphins.)

BETA-ENDORPHIN has been measured in the plasma of healthy human volunteers and in patients suffering from pituitary-adrenal disease.[1-3] While there seems to be little doubt that beta-endorphin resembles morphine pharmacologically and will bind with opiate receptors along the principal routes of pain stimuli, opiate receptors have also been identified in areas not associated with pain but with emotional responses and with hormone control.[4-7] That stressful conditions may play a role in generating endorphin was recently documented by Wardlaw and co-workers[8] who reported that plasma levels of beta-endorphin and beta-lipotropin (beta-LPH) obtained from human fetuses at term are inversely correlated with arterial *p*H and P_{O_2}. This observation suggested that hypoxia and acidosis may be important physiological stimuli for the release of beta-endorphin and beta-LPH in the human fetus. In the present study we attempted to determine whether adults respond similarly to the human fetus by measuring plasma levels of beta-endorphin-like immunoreactivity in patients suffering from hypoxia and acidosis for which they were being treated in respiratory care units (RCU). In addition, we attempted to correlate beta-endorphin-like immunoreactivity in the hypoxic patients with arterial pH and P_{O_2}. Healthy adult volunteers served as controls.

Materials and Methods

In accordance with existing rules and regulations observed and enforced by the Human Use Committee of Tokyo Kosei Nenkin Hospital, the participants in this study, patients as well as volunteers, were familiarized fully with the objectives of this investigation and their right to refuse to participate in the project if they so desired. All participants signed an informed consent.

Twenty male adult patients suffering from hypoxia and acidosis of various etiologies were admitted to the respiratory care unit (RCU) where they received respiratory support including oxygen inhalation by mask, mechanically controlled or assisted respiration, PEEP, CPAP, and/or IMV. Patients with painful disorders or with hormonal dysfunction were excluded from the study. CNS or pituitary stimulants or depressants were omitted for 10 hours preceding blood samplings.

In another twenty healthy male volunteers‡ with an average age of 60 ± 2 (SEM) years and with normal endocrine function, Pa_{O_2}, Pa_{CO_2}, and *p*H were determined and found to range from 82–95 torr, 38–43 torr, and 7.38–7.42, respectively. Arterial blood samples were collected from both patients and volunteers during the morning hours (8 A.M. to 10 A.M.).

Blood was collected in heparinized tubes, immediately centrifuged at 4°C and stored frozen at −20°C if not assayed immediately. Periods of frozen storage (up to 1 week) did not alter human beta-endorphin ($beta_h$-endorphin) concentrations. Plasma was extracted, chromatographed, and assayed for beta-endorphin employing the method reported by Wardlaw and colleagues,[9] as follows:

Plasma Extraction. 10 to 20 ml of plasma was extracted with one 50-mg talc tablet per 5 ml of plasma. The absorbed $beta_h$-endorphin was eluted from the talc with 1 ml of 1:1 acetone-0.1 N HCl per 50 mg talc. The acetone-HCl extracts were evaporated under a stream of air at 20°C and assayed directly for total beta-endorphin-like immunoreactivity. The residue was either dissolved in 0.5–1.0 ml of the assay buffer for direct assay or in 0.1 N acetic acid containing 0.1 per cent bovine serum albumin (BSA) for chromatography.

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‡ All volunteers were active members of an amateur marathon club and ran 3–5 km daily.

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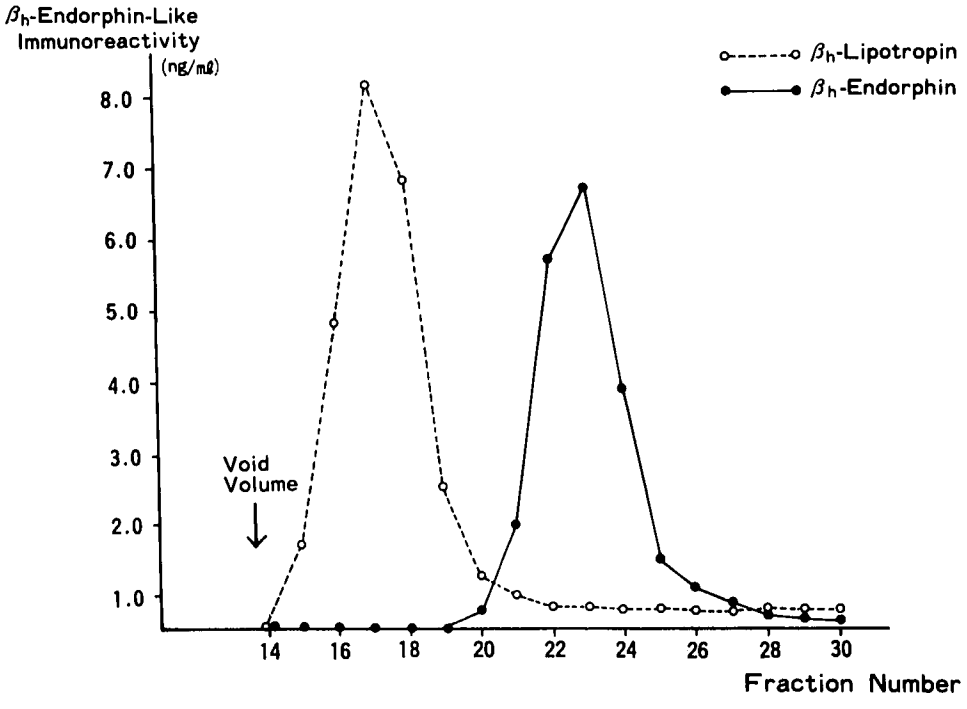

FIG. 1. Elution pattern of $beta_h$-endorphin and $beta_h$-LPH standards.

Antiserum. 0.5 mg $beta_h$-endorphin was conjugated to 2 mg of thyroglobulin with 50 mg of carbodiimide.[10] Rabbits were immunized monthly with 0.8 mg of the conjugate in complete Freund's adjuvant. The antiserum used in this study bound 40 per cent of the tracer at a final dilution of 1:60,000.

Iodination. Beta-endorphin was labeled with ^{131}I by the lactoperoxidase method.[11] The tracer was purified by Sephadex G-50 chromatography (1.7 × 30 cm) in 0.04 M *p*H 7.5 phosphate buffer containing 0.05 M NaCl and 0.5 per cent BSA.

Chromatography. 20-ml plasma samples were ex-

TABLE 1. Age, Disease, Arterial *p*H and P_{O_2}, Beta-Endorphin-Like Immunoreactivity in 20 Patients

Patient	Age	Disease	*p*H	P_{O_2} (torr)	Beta-Endorphin-Like Immunoreactivity (pg/ml)
1	56	Thoracic trauma	7.35	79	15.1
2	56	Aspiration pneumonitis	7.35	79	20.3
3	62	Aspiration pneumonitis	7.29	74	20.5
4	78	Atelectasis	7.34	76	30.0
5	63	Atelectasis	7.27	69	34.0
6	51	Postgastrectomy	7.33	76	41.2
7	56	ARDS	7.25	63	33.1
8	67	Aspiration pneumonitis	7.27	64	42.3
9	45	Post-open heart surgery	7.29	68	48.5
10	49	Lung cancer	7.30	73	58.6
11	67	ARDS	7.24	69	47.9
12	71	ARDS	7.18	59	56.1
13	70	ARDS	7.28	69	60.0
14	66	Postgastrectomy	7.22	68	59.1
15	62	Postgastrectomy	7.18	56	66.3
16	70	ARDS	7.24	65	73.9
17	72	ARDS	7.17	54	80.0
18	63	Asthma	7.16	63	88.5
19	64	Asthma	7.15	59	88.0
20	50	Post-Open heart surgery	7.14	49	100.2
MEAN ± SEM	61 ± 2		7.25 ± 0.02	67 ± 2	53.2 ± 5.5

See text.

TABLE 2. Differences of Beta-Endorphin-Like Immunoreactivity between an Improved State and a Hypoxic State

Patient	Date	pH	P_{O_2} (torr)	Beta-Endorphin-Like Immunoreactivity (pg/ml)
7	April 2, 1979	7.25	63	33.1
	April 19, 1979	7.38	83	4.2
8	August 10, 1979	7.27	64	42.3
	September 1, 1979	7.41	89	6.0
15	September 1, 1979	7.18	56	66.3
	September 22, 1979	7.40	89	3.9
17	June 21, 1979	7.17	54	80.0
	July 10, 1979	7.39	76	12.9
19	March 6, 1980	7.15	59	88.0
	March 9, 1980	7.38	80	40.8

Patients 7, 8, 15, 17, and 19 are the same as indicated in table 1.

tracted as described above and chromatographed on 0.9 × 45 cm Sephadex G-50 (fine) columns in 0.1 N acetic acid with 0.1 per cent BSA in order to assay beta-endorphin and beta-LPH separately. Recovery for added beta-endorphin and beta-LPH after plasma extraction averaged 75 per cent (range, 71–80 per cent) for both peptides. Overall recovery after extraction and chromatography was 68 per cent (range, 63–74 per cent). Elution from 0.9 × 45 cm Sephadex G-50 column gives a sharp separation of $beta_h$-endorphin and $beta_h$-LPH standards (fig. 1). The measured $beta_h$-endorphin concentration is the total immunoreactivity contained in fraction 21–26, corresponding to the elution of $beta_h$-endorphin standard.

Assay. Total beta-endorphin-like immunoreactivity was measured by radioimmunoassay using synthetic $beta_h$-endorphin standard and an antiserum to human beta-endorphin. This antiserum cross-reacts with human beta-LPH, which has 10.8 per cent the immunoreactivity of beta-endorphin on a weight basis. Current assay sensitivity is 10 pg/tube. Synthetic $beta_h$-endorphin standards (25–1000 pg) or sample extracts (50–200 ul) were pre-incubated with antibody (final concentration 1:5000) and carrier rabbit gamma-globulin (50 μg) in a total of

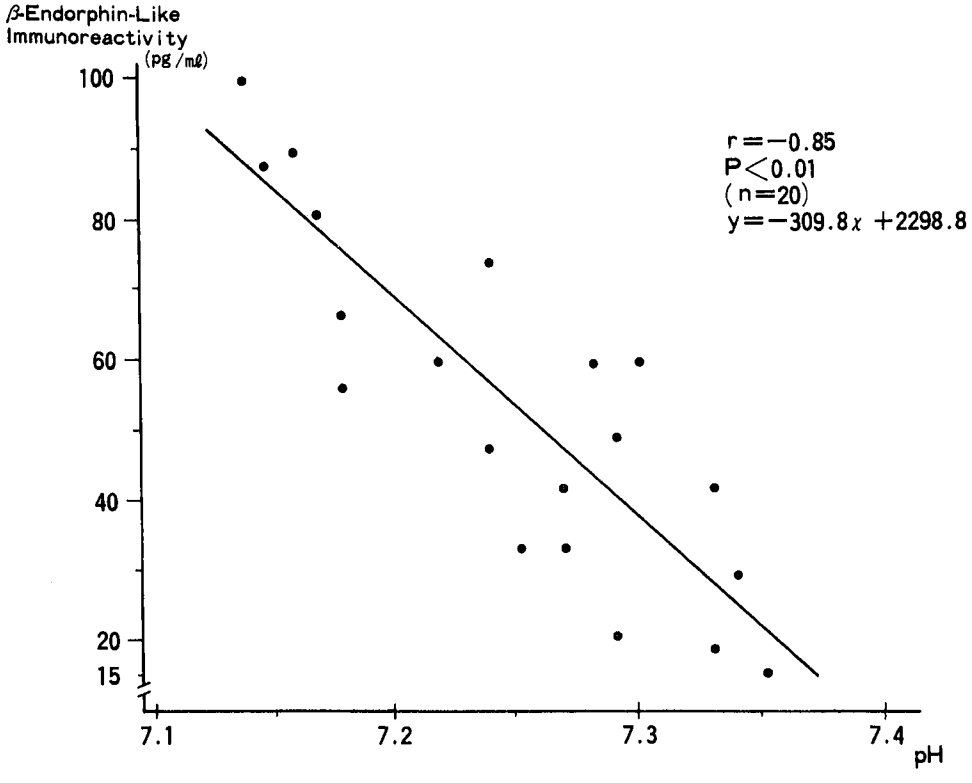

FIG. 2. Correlation of beta-endorphin-like immunoreactivity with arterial pH in 20 hypoxic patients.

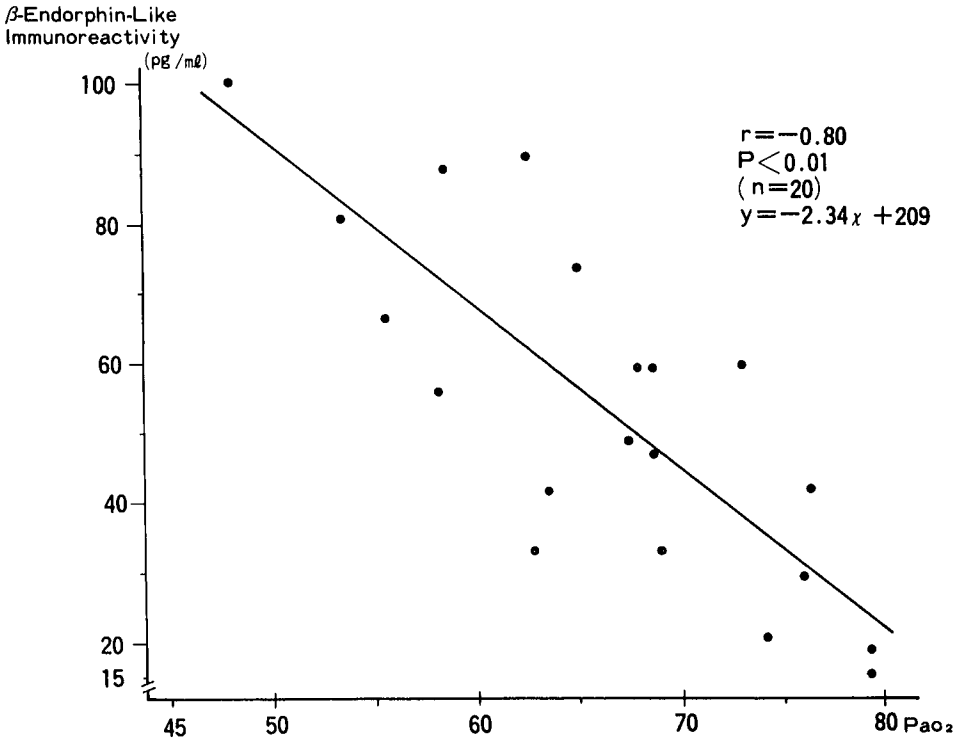

FIG. 3. Correlation of beta-endorphin-like immunoreactivity with Pa_{O_2} in 20 hypoxic patients.

4.5 ml assay buffer (0.04 M *p*H 7.5 phosphate with 0.05 M NaCl and 0.5 per cent BSA) for 24 h at 4° C. Fifty microliters of ^{131}I-beta$_h$-endorphin was then added and incubation continued for 24 h at 4° C. Bound and free peptides were separated by precipitation with sheep anti-rabbit gamma-globulin. Parallelism of patients plasma extracts with synthetic beta-endorphin standard was demonstrated over the effective range of the standard curve.

We expressed all values of age of hypoxic patients, arterial *p*H, Pa_{O_2}, and beta-endorphin-like immunoreactivity as mean ± SEM. Beta-endorphin-like immunoreactivity from hypoxic patients were compared with volunteers by Student's *t* test. Differences with $P < 0.01$ were considered significant. Pa_{O_2}-beta-endorphin-like immunoreactivity relations as well as arterial *p*H-beta-endorphin-like immunoreactivity relations were analyzed with linear regressions by the least-squares method.

Results

The mean age of the patients was 61 ± 2 years. Pa_{O_2}, Pa_{CO_2}, and *p*H in these patients ranged from 49–79 torr, 38–46 torr, and 7.14–7.35, respectively. Table 1 lists the patients as to age, disease, *p*H, Pa_{O_2} and beta-endorphin-like immunoreactivity (pg/ml).

Mean beta-endorphin-like immunoreactivity in the plasma of the volunteers was 6.2 ± 1.9 pg/ml, as compared to 53.2 ± 5.5 pg/ml measured in the patients, ($P < 0.01$, table 1). In five patients beta-endorphin-like immunoreactivity decreased at an improved state of arterial *p*H and P_{O_2} as compared with the hypoxic state (table 2). Negative correlations were present between beta-endorphin-like immunoreactivity concentration and arterial *p*H ($r = -0.85$; $P < 0.01$, fig. 2) and P_{O_2} ($r = -0.80$; $P < 0.01$, fig. 3).

Discussion

The physiological significance of increased levels in plasma beta-endorphin is poorly understood. However, the well-documented release of beta-endorphin and ACTH from the pituitary gland in response to hypoglycemia and electroshock stimulation or in connection with the administration of metyrapone and vasopressin has been interpreted as a stress response.[1-3,8,12-14] Hypoxia has recently been found to cause an increase in ACTH,[15] and beta-endorphin in the plasma of the sheep fetus.§ High levels of circulating beta-endorphin-like immunoreactivity have also been detected in the human fetus at term.[8] Our findings of markedly increased beta-en-

§ Wardlaw SL, Stark RI, Frantz AG: unpublished observations.

dorphin-like immunoreactivity in adult patients suffering from hypoxia and acidosis seem to confirm that this condition represents a stressful stimulus which causes an increase of beta-endorphin-like immunoreactivity. Further, beta-endorphin-like immunoreactivity may be involved in a physiologic response to stress.

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References

1. Krieger DT, Liotta AS, Suda T, et al: Human plasma immunoactive lipotropin and adrenocoricotropin in normal subjects and in patients with pituitary-adrenal disease. J Clin Endocrinol Metab 48:566–571, 1979
2. Wiedemann E, Saito T, Linfoot JA, et al: Radioimmunoassay of human beta-lipotropin in unextracted plasma. J Clin Endocrinol Metab 45:1108–1111, 1977
3. Jeffcoate WJ, Rees LH, Lowry PJ, et al: A specific radioimmunoassay for human beta-lipotropin. J Clin Endocrinol Metab 47:160–167, 1978
4. Barchas JD, Akil H, Elliott GR, et al: Behavioral neuro-chemistry, neuroregulators and behavioral neurochemistry: neuroregulators and behavioral states. Science 200:964–973, 1978
5. Frederickson RCA: Minireview—Enkephalin pentapeptides: A review of current evidence for a physiological role in vertebrate neurotransmission. Life Sci 21:23–42, 1977
6. Janowsky DS, Segal DS, Bloom F, et al: Lack of effect of naloxon on schizophrenic symtoms. Am J Psychiatry 134:926–927, 1977
7. Bunney WE, Pert CB, Klee W, et al: Basic and clinical studies of endorphins. Ann Intern Med 91:239–250, 1979
8. Wardlaw SL, Stark RI, Baxi L, et al: Plasma beta-endorphin and beta-lipotropin in human fetus at delivery: correlation with arterial pH and P_{O_2}. J Clin Endocrinol Metab 79:888–891, 1977
9. Wardlaw SL, Fantz AG: Measurement of beta-endorphin in human plasma. J Clin Endocrinol Metab 48:176–180, 1979
10. Goodfriend TL, Levine L, Fasman GD: A use of carbodiimides in immunology. Science 144:1344–1346, 1964
11. Thorell JL, Johansson BG: Enzymatic iodination of polypeptides. Biochim Biophys Acta 251:363–369, 1971
12. Nakao K, Nakaki Y, Oki S, et al: Presence of immunoreactive beta-endorphin in normal human plasma. J Clin Invest 62:1395–1398, 1978
13. Rossier J, French ED, Rivier C, et al.: Foot-shock induced stress increases beta-endorphin levels in blood but not brain. Nature 270:618–620, 1977
14. Haller EW, Sachs H, Sperelakis N, et al: Release of vasopressin from isolated guinea pig posterior pituitaries. Am J Physiol 209:79–83, 1965
15. Boddy K, Jones CT, Mantell C, et al: Changes in plasma ACTH and corticosteroid of the maternal and fetal sheep during hypoxia. Endocrinology 94:588–593, 1974