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A STUDY OF THE ENDOGENOUS OPIOID SYSTEM IN THE SUDDEN INFANT DEATH SYNDROME

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ABSTRACT

To investigate the possible role of the endogenous opioid system in the pathogenesis of the sudden infant death syndrome (SIDS), we measured met-enkephalin immunoreactivity by radioimmunoassay in brain, lung, and adrenal glands of SIDS victims and of infants (controls) dying of nonneurologic causes. Met-enkephalin was stable in brain tissue up to 24 hours after death. On inspection, met-enkephalin levels in the cerebral cortex of SIDS victims were similar to those in controls. Levels in the caudate nucleus were lower in infants than in adults. In the medulla, the levels in SIDS cases and controls were not found to differ significantly. The linear relationship between the levels in the medulla and age was not detectably different in SIDS and controls. However, as a subset, levels in the control group significantly decreased with increasing age ($P = 0.005$), whereas levels in the SIDS group showed no correlation with age ($P = 0.33$). Levels of met-enkephalin in the adrenal gland of SIDS victims were similar to those in controls and were considerably lower than adult values. Lung tissue was assayed for beta-endorphin immunoreactivity and met-enkephalin: for both peptides the values in SIDS cases were low (femtomolar range) and similar to those in controls. These data suggest that met-enkephalin is not markedly overproduced in brains of SIDS victims. Future postmortem studies should focus on more subtle evidence of endogenous opioid overactivity such as differences in age related changes, receptor number, and levels of other endogenous opioid peptides.

INTRODUCTION

Each year in the United States 7,000 to 8,000 lives are lost as a result of the sudden infant death syndrome (SIDS).[1] SIDS is the sudden, unexpected death of an apparently healthy infant in whom a routine autopsy fails to reveal the cause of death. It usually occurs while the infant is thought to be asleep. In the

United States and many developed countries, SIDS is the most common cause of death among infants between one month and 12 months of age.[2] Considerable evidence suggests that most SIDS victims were hypoxemic for weeks or months before death.[1,3,4] It is now generally agreed that abnormal ventilatory control, leading to protracted apnea, especially during sleep, may be part of the pathogenic mechanism in many instances of SIDS.[3,5] Current research strategies, therefore, have focused on the neural regulation of ventilation during infancy [6,7] Some investigators have suspected that a neurochemical abnormality may be the cause of the abnormal ventilatory control.[8,9] However, few studies of neurochemistry have been done in SIDS.[3]

The unconsciousness, respiratory depression, and fatal respiratory arrest that result from morphine overdose[10] and the knowledge that therapeutic doses of morphine significantly depress hypoxic and hypercapnic ventilatory drives in man[11] led investigators to study the effects of the endogenous opioid peptides on ventilation. The endogenous opioid peptides (met-enkephalin, leu-enkephalin, beta-endorphin, and others) are commonly referred to as endorphins ("endogenous morphine"). These peptides exert opiatelike actions by way of specific interactions with opioid receptors. They are present in brain, pituitary gland, adrenal gland, plasma, and other tissues.[12-14] Indeed, although several studies indicate that endorphins are not involved in the regulation of ventilation in normal adult humans,[15-17] they cause respiratory depression, decreased ventilatory drive,[18-19] and apnea[20] in animals and ventilatory disorders in some pathophysiologic states in man.[21,22]

The unique characteristics of SIDS, in particular the quiet, sudden death during presumed sleep,[9,23] led us to investigate the possibility that overactivity of the endogenous opioid system may cause SIDS.[24] To our knowledge, this is the first reported study of the endogenous opioid system in SIDS.

METHODS

Collection of Tissue. Postmortem tissue from SIDS victims and control infants was collected (by T.E.K.) with the assistance of Dr. Haruo Okazaki and the pathology staff of the Mayo Clinic, Rochester, Minnesota, and by Dr. Ralph Franciosi, Children's Health Center and Hospital, Minneapolis, Minnesota. One of us (T.E.K.) also collected cases from the Cook County Morgue with the cooperation of Dr. Robert J. Stein, Chief Medical Examiner, Cook County, Chicago, Illinois. All autopsies were done within 24 hours of death except in one case.

Control Cases. The control cases were infants between the ages of two weeks and 12 months who died from accidental causes or congenital cyanotic heart disease and who had grossly normal brains at autopsy. The cases of congenital cyanotic heart disease serve as examples of known chronic hypoxemia and will be referred to as "hypoxic controls."

Handling. The fresh, unfixed tissue was dissected at the time of autopsy and deep frozen at -70°C until analysis. Samples were taken from brain, lung (right upper lobe), and adrenal gland. In some cases, one cerebral hemisphere (the left in most cases) was frozen whole and stored at -70°C. Dissection was subsequently done in a cold room at 4°C after the cerebral hemisphere was allowed to thaw at 4°C; it was dissected while still firm. The tissue sections (kept on ice) were then immediately extracted.

Stability Study. For assessment of the stability of met-enkephalin in postmortem human brain tissue, both caudate nuclei were removed from one brain within three hours of the time of death. One small piece from one of the caudates was immediately frozen, and the remaining piece was refrigerated at 2 to 4°C. The other caudate was left at room temperature (21 to 22°C). At 5, 8, 12, 19, and 24 hours after the time of death, a small slice from each caudate was frozen and stored at -70°C until analysis.

Tissue Extraction Procedure for Met-Enkephalin and Beta-Endorphin. The deep-frozen tissue samples were allowed to thaw at 4°C, weighed, added to plastic test tubes containing 10 volumes of 1 M acetic acid (which had been preheated to 98°C), and heated at 98°C for 15 minutes.[25,26] The solution was cooled on ice for a minimum of 10 minutes and then homogenized at 0°C (Polytron homogenizer, setting 7, 30 seconds). The homogenate was centrifuged at 10,000 x g for 20 minutes at 4°C.[25] At this point an aliquot of the supernatant was lyophilized and stored at -20°C for future beta-endorphin determination. The remainder of the supernatant was applied to a C-18 column (Sep-Pak cartridge) that had been prewashed with 5 ml of absolute methanol and 12 ml of water. Then the column was washed with 4 ml of 1 M acetic acid and 4 ml of water and the eluates were discarded. The met-enkephalin-like material was eluted from the column with 4 ml of absolute methanol, which was subsequently removed by negative pressure on the Evapo-Mix device (modified method of Gilbert and associates).[27] The residue, a crude tissue extract containing enkephalins, was stored at -20°C until analysis. Plasticware or silanized glassware was used for all the above steps.

For an estimate of the recovery of met-enkephalin-like material from the tissue by the extractive procedure, 2 to 4 ng of synthetic met-enkephalin was separately processed in parallel with tissue samples, and the amount recovered was measured by radioimmunoassay. Interassay variability was determined by (1) repeated extraction and radioimmunoassay of remaining frozen tissue that was not used in the initial extraction; and (2) resuspension of some of the stored lyophilized supernatants and application to a C-18 column followed by radioimmunoassay for met-enkephalin.

Radioimmunoassay. The amount of met-enkephalin or beta-endorphin immunoreactive material in the tissue extracts was determined by radioimmunoassay performed with met-enkephalin kits and beta-endorphin kits from Immuno Nuclear Corporation, Stillwater, MN. Triplicate radioimmunoassay determinations were done on each

extract (quadruplicates were done for some extracts). The amount of ^{125}I-labeled met-enkephalin bound to antibody in the reaction precipitates was measured (LKB 1275 mini gamma counter). The best fit for the standard curve and the potency estimates for each unknown sample were computed.[27]

The radioimmunoassay for met-enkephalin was reported to have 1.6% cross-reactivity for leu-enkephalin. Cross reactivities for beta-endorphin, substance P, gamma endorphin, and alpha-endorphin are all less than 0.01%. The sensitivity was 8 pg (14 fmol).

The beta-endorphin measurements in lung tissue were performed on the lyophilized supernatants resuspended in radioimmunoassay buffer on ice. Beta-lipotropin, which cross reacts with the anti-beta-endorphin antibody, was stripped from the sample by affinity gel extraction that utilized a sepharose-antibody complex specific for the N-terminal of beta-lipotropin. The amount of ^{125}I-labeled beta-endorphin bound to antibody in the reaction precipitates was measured, and the data were handled in the same manner as for met-enkephalin.

The radioimmunoassay for beta-endorphin was reported to have 100% cross reactivity with both DES-Tyr^1-human beta-endorphin and 2-Me-Ala^2-beta-endorphin, and 50% cross reactivity with human beta-lipotropin. Cross reactivities with leu-enkephalin, met-enkephalin, ACTH, dynorphin, alpha-endorphin, and gamma-endorphin are all less than 0.1%. The sensitivity was 2 pg (1 fmol).

Since no extensive chemical characterization was done, the measured activity can only be attributed to "radioimmunoassayable material."

For two-sample comparisons, both the two-sample t test and the rank-sum test were performed. For paired or one-sample comparisons, the paired t test and the signed-rank test were used. The two-tail level of significance was taken as the conventional 0.05. When significance differed, depending on the statistical procedure, both results are reported; otherwise, for brevity, only the t test results are noted if they agreed.

RESULTS

Extraction Procedure and Radioimmunoassay. The percent recovery of met-enkephalin by our extraction procedure was 80 to 90% ($n = 4$) as determined by radioimmunoassay. The triplicate radioimmunoassay dilutions were within 10% of their mean. Inter-assay variability was less than 10%.

Confirmation of Cases. The final results of the autopsies and postmortem studies by the pathologist in all the cases of SIDS confirmed the diagnosis of SIDS (that is, results of toxicologic, bacteriologic, and viral studies were negative).

Stability of Met-Enkephalin in Postmortem Tissue. The met-enkephalin content in human caudate tissue obtained within three hours after the time of death was 820 ± 50/pmol/g wet weight (X ± SD). This was not significantly different from the content in tissue that was left at room temperature (21°C) for 19 hours after death (820 ± 105 pmol/g), in tissue 24 hours after death (1,097 pmol/g), or in tissue refrigerated for 21 hours at 4°C (beginning three hours after death) 738 ± 148 pmol/g (Fig. 1).

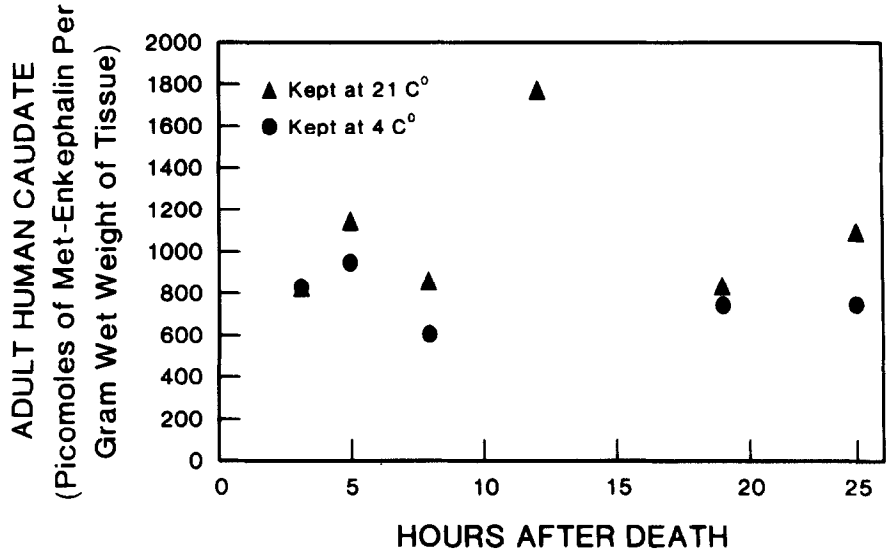

Fig. 1 Met-enkephalin levels in human caudate tissue in relation to time after death. Earliest point is 3 hours after death. Each point represents concentration in a single slice of tissue. High point at 12 hours in sample kept at room temperature may be due to regional difference in particular slice of tissue assayed.

The high value (1,893 pmol/g) in the sample kept at 21°C (the 12-hour sample) may represent a regional difference in the particular slice of caudate which was assayed, since one piece of tissue was used for each point. These data are consistent with the remarkable stability of enkephalin noted by Emson and colleagues,[28] who demonstrated no significant change in met-enkephalin content for up to 72 hours after death in mouse brains that were gradually cooled in a manner designed to simulate normal postmortem handling of human brains.

Met-Enkephalin in Adult Brain. To check our extraction procedure, we studied adult brain tissue because the regional distribution of met-enkephalin content in adult brain had been mapped out by two different groups.[25,28] Our results were similar to those that have been reported in both absolute values and regional distribution (Table 1). Highest levels of met-enkephalin were found in the caudate nucleus, globus pallidus, and substantia nigra. Much lower values were found in the cortex and subcortex.

Table 1
Adult Brain Met-Enkephalin Levels
(Picomoles of Met-Enkephalin per Gram Wet Weight of Tissue)*

	Present Study	Emson et al.[28]	Gramsh et al.[25]
Cortex	10-21 (3)	42 (16)	Very low
Caudate nucleus	447-1,120 (2)	116 (20)**	1,000 - 2,000 (4)
Globus pallidus	260-500 (2)	1,163 (40) (lateral) 675 (40) (medial)	1,000 - 2,000 (5)
Substantia nigra	260 (1)	557 (15) (pars compacta) 661 (15) (pars reticulata)	1,000 (4)

*Numbers in parentheses are numbers of cases.
**This low value may reflect a regional anatomic difference due to dissection technique.

Met-Enkephalin in Infant Brain. Generally, the values in the five brain areas studied varied considerably (except the medullas) in both SIDS victims and controls (Table 2). Interpretation is made more difficult by the fact that there is only one report of these values in childhood,[22] and values for infants less than one and a half years old have not been previously reported. On inspection, the values did not show any apparent relationship to sex, race, or source of the case. Generally, values in SIDS and controls were similar. In the cortex, no apparent changes occurred with age (n = 8). Levels in the caudate were lower than values in adults by a factor of about 10 but were similar to the values in children one and a half to two years old reported by Brandt and associates.[22]

Met-Enkephalin in Infant Medullas. The average met-enkephalin content in the medullas of the SIDS group was 32 pmol/g tissue (SD = 14, n = 14). This was not significantly different from all controls--31 pmol/g (SD = 23, n = 6)--or from the hypoxic control group--42 pmol/g (SD = 17, n = 4). The average met-enkephalin content in the medullas of the SIDS group was significantly higher than that of the trauma controls, which was 8 pmol/g (SD = 4, n = 2), $P = 0.033$; however, this difference is not interpretable because of the older age (11 months and 12 months) and small number (n = 2) of the trauma controls (Table 3).

Table 2

Infant Brain Met-Enkephalin Levels

(Picomoles of Met-Enkephalin per Gram Wet Weight of Tissue)

Age (mo)	1	1-1/2	3	3-1/2	5	10	10	11	12	
SIDS or control	SIDS	SIDS	SIDS	SIDS	SIDS	Control	SIDS	Control	Control	Control**
Sex/race	F/W	F/W	M/B	M/W	F/W	M/W	F/B	F/B	M/W	
Source of case*	C.C.	Mpls.	C.C.	Olm	C.C.	Mayo	C.C.	C.C.	C.C.	
Case no.	3	4	8	10	13	17	18	19	20	
Petechiae present on thymus or pleura	Yes	Yes	No	Yes	No	No	Yes	No	No	
Cortex (frontal)	29	12	11	37	8	11	---	3	1	$\leq$ 25 (2)
Subcortex (white matter)	---	37	---	10	---	6	---	---	---	25-50 (3)
Caudate	---	48	16	4	10	263	---	47	451	"Corpus striatum" (1) 30
Periaqueductal gray	---	27	---	85	---	---	---	---	---	
Medulla (whole or average of medial and lateral sections)	34	35	26	61	16	28	34	5	11	

*C.C. = Cook County, Chicago, Illinois; Mpls. = Minneapolis, Minnesota; Olm = Olmsted County, Minnesota; Mayo = case referred to Mayo Clinic.

**Values reported by Brandt and associates[22] for three controls: 1-1/2-year-old, 2-year-old, and 2-year-old (congenital heart disease); numbers in paretheses = number of control cases.

Table 3

Levels of Met-Enkephalin in Medullas From 20 Infants

(Picomoles of Met-Enkephalin per Gram Wet Weight of Tissue)*

Case	Age (mo)	Cause of death	Sex/race	Source of case**	Petechiae present on thymus or pleura	Levels		
						Medial medulla	Lateral medulla	Whole medulla (or average of medial and lateral values)
1	1/2	CCHD***	M/W	Mayo	No	65	65	65
2	3/4	SIDS	M/W	C.C.	---	41	39	40
3	1	SIDS	F/W	C.C.	Yes	27	40	34
4	1-1/2	SIDS	F/W	Mpls	Yes	---	---	35
5	2	SIDS	F/W	Olm	Yes	60	46	53
6	2-1/2	SIDS	F/W	Olm	Yes	28	27	28
7	3	SIDS	F/W	C.C.	No	39	36	38
8	3	SIDS	M/B	C.C.	No	27	25	26
9	3-1/4	SIDS	M/W	Mpls	Yes	19	12	16
10	3-1/2	SIDS	M/W	Olm	Yes	---	---	61
11	4	CCHD|	F/W	Mayo	---	41	47	44
12	5	CCHD||	F/W	Mpls	No	39	23	31
13	5	SIDS	F/W	C.C.	No	16	16	16
14	6	SIDS	M/W	Mpls	Yes	28	24	26
15	6	SIDS	M/W	Mpls	Yes	11	10	11
16	7-1/2	SIDS	M/W	Mpsl	Yes	37	25	31
17	10	CCHD|||	M/W	Mayo	No	---	---	28
18	10	SIDS	F/B	C.C.	Yes	36	33	34
19	11	Apparent drowning	F/B	C.C.	No	---	---	5
20	12	Gunshot wound	M/W	C.C.	No	11	11	11

In the 16 cases in which the met-enkephalin content of the medial and lateral sides of the medulla was determined, the tendency (14 of 16) was for the medial medulla content to be equal to or greater than that of the lateral medulla. This reached statistical significance when the signed-rank test ($P = 0.032$) was used but not with the paired t test. In nine cases the difference between the medial and the lateral medulla was only 3 pmol or less (Table 3).

Met-Enkephalin in Adrenal Tissue. Small amounts of met-enkephalin were present in sections from the adrenal gland (containing tissue from both the adrenal cortex and the adrenal medulla). Two SIDS cases, one and a half months and three and a half months of age, had values of 18 and 1 pmol/g wet weight of tissue, respectively. Two controls with congenital cyanotic heart disease, three and a half months and nine months of age, had values of 8 and 0.6, respectively. A pronounced difference between SIDS cases and control cases was not apparent, and these values are much lower than the few values that have been reported in adults.[14] There are no reports of met-enkephalin levels in infant adrenal glands available for comparison.

Beta-Endorphin and Met-Enkephalin in Lung Tissue. Small amounts (femtomolar range) of beta-endorphin and met-enkephalin were present in infant lung tissue (Table 4). Although we did not specifically study the stability of beta-endorphin, in the seven cases in which it was assayed, the values were all very similar and did not show any relationship to the elapsed time between death and autopsy. This finding is consistent with a previous report which also suggests that beta-endorphin immunoreactive material is stable with respect to time after death.[29] We cannot rule out the possibility however, that these low values may represent a postmortem artifact.

Table 4
Beta-Endorphin and Met-Enkephalin in Lung Tissue*

Age (mo)	Case no.	Diagnosis	Levels	
			Beta-Endorphin	Met-Enkephalin
1-1/2	4	SIDS	---	11,000
3	8	SIDS	428	---
3-1/2	10	SIDS	432	760
3-1/2	--	CCHD	---	520
5	13	SIDS	439	---
10	17	CCHD	497	580
10	18	SIDS	639	---
11	19	Apparent drowing	315	---
12	20	Gunshot wound	493	---

CCHD = Congenital Cyanotic Heart Disease.
*All values are in femtomoles per gram wet weight of tissue, ± approximately 20%.

When the concentration of met-enkephalin in the medullas of all infants studied (n = 20) was compared with respect to age, the concentration was found to decrease significantly with increasing age ($P = 0.008$). The linear relationship between the levels in the medulla and age was not detectably different in SIDS and controls. However, as a subset, the met-enkephalin concentration in the medullas of the SIDS cases (n = 14) showed no change with respect to age ($r = -0.28$, $P = 0.33$), whereas the control group (n = 6) showed a significant ($r = -0.94$, $P = 0.005$) decrease with increasing age (Figure 2).

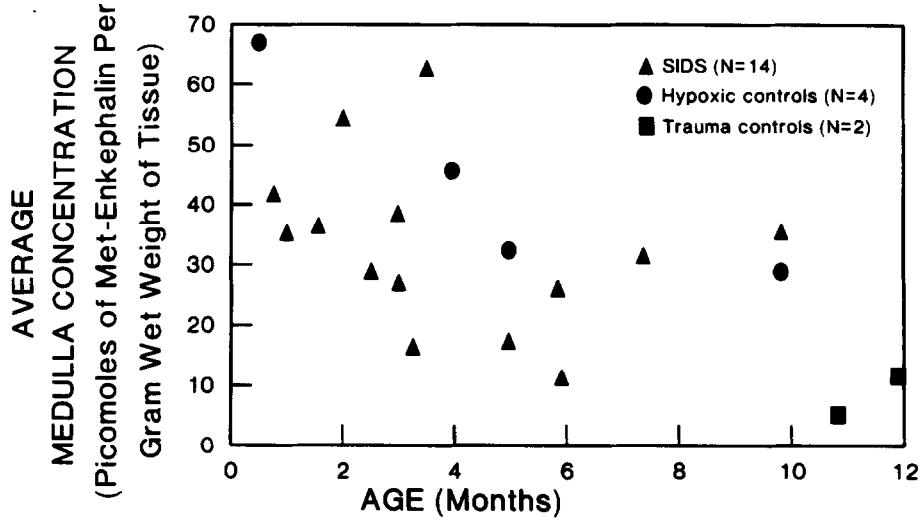

Fig. 2 Scatter diagram of average medulla met-enkephalin concentration versus age.

LEGEND FOR TABLE 3

*Total number of SIDS cases = 14 (7 boys, 7 girls); total number of control cases = 6: 4 hypoxic controls (2 boys, 2 girls) and 2 trauma controls (1 boy, 1 girl); CCPH = congenital cyanotic heart disease.

**C.C. = Cook County, Chicago, Illinois; Mpls = Minneapolis, Minnesota; Olm = Olmsted County, Minnesota; Mayo = case referred to Mayo Clinic.

***Died 15 minutes after surgery for total anomalous pulmonary venous return; anesthetics were pancuronium bromide and fentanyl; had low P_{O_2} before death.

¶Died 6 days after surgery for complete atrioventricular canal; sustained cardiac arrest and as a result suffered a severe anoxic insult; pupils were fixed and dilated; had spontaneous respirations but no other detectable neurologic function.

¶¶Complete abdominal and thoracic situs inversus, double-outlet right ventricle; did not have surgery.

¶¶¶Died 4 days after surgery for complete transposition of the great vessels; low P_{O_2} before death; neuropathology revealed acute hypoxic encephalopathy.

The one-and-a-half-month-old SIDS case had a very high level of met-enkephalin in comparison with the three other cases; however, such a value was not seen in the three-and-a-half-month-old SIDS case, and it may reflect an age-related change. There are no reports of levels of these peptides in human lung tissue available for comparison.

DISCUSSION

A considerable advance in our understanding of SIDS came in 1973 when Naeye[30] reported that smooth muscle fibers in small pulmonary arteries were commonly both hyperplastic and hypertrophied in this condition. There is evidence suggesting that this finding and other subtle tissue abnormalities noted in SIDS[1] are a result of chronic hypoxemia, which may be present weeks or months before death from SIDS.[1,4] Studies of infants deemed "near-miss" for SIDS* also suggest that abnormal ventilatory control may be a factor in the pathogenesis of SIDS.[31-34]

These findings and other clinical observations are consistent with the apnea hypothesis of SIDS proposed by Steinschneider[35] in 1972 and have recently been well reviewed.[3,5,9,36,37] Most investigators agree that protracted apnea, especially during sleep, plays a role in many cases of SIDS.[3,5,9] However, the cause of the apnea and the subsequent silent, sudden death is unknown.[3,36]

Clinically imperceptible hypoventilation with substantial depression of hypercapnic and hypoxic ventilatory drive has been reported in conscious, awake men after administration of only 7.5 mg of morphine subcutaneously.[11] Thus, it was not surprising when, in 1977, Florez and Mediavilla[38] demonstrated that an endogenous opioid peptide (met-enkephalin) produced a naloxone-reversible (naloxone blocks the effects of endogenous opioid peptides) respiratory depression when applied to the ventral surface of the cat brain, an effect subsequently noted to be elicited by beta-endorphin as well.[39] Moss and associates demonstrated in 1978 that elevation of beta-endorphin levels by direct injection into the cisterna magna of adult dogs produced a naloxone-reversible respiratory depression and a reduced central responsivity to carbon dioxide.[18,40] Studies also suggest that endogenous opioid peptides may be involved in the pathogenesis of apnea.[20,22,41]

In humans, several studies have indicated that endogenous opioid peptides are not involved in the regulation of ventilation in normal adults.[15-17] However, participation of the endogenous opioid system in the control of breathing in unanesthetized human beings with obstructive airway disease has recently been demonstrated.[21,42]

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*These are infants noted by a caretaker to be apneic, limp, and pale or cyanotic who require vigorous shaking or cardiopulmonary resuscitation to restore respirations and for whom a subsequent rigorous medical evaluation fails to reveal the cause of the episode.[9]

Kuich and Zimmerman[24,43] have proposed that SIDS and near-SIDS may be caused by overactivity of the endogenous opioid system. In addition to respiratory depression and apnea, the endorphin overactivity as shown by Brandt and associates[22] may also provide an element of supraspinal analgesia and thus account for a quiet death during presumed sleep.

We found that in cortex, subcortex, caudate nucleus, medulla, lung, and adrenal tissue there were no pronounced differences in met-enkephalin levels in cases of SIDS in comparison with controls (Table 2). Levels in the cortex, subcortex and caudate nucleus varied widely between cases. The values were not remotely close to the very elevated levels found in the hyperendorphin case reported by Brandt and associates,[22] but were similar to the control values which they reported (Table 2). Within a given brain, values in different regions were variously increased or decreased in comparison with the same regions in other brains. This indicates that in SIDS there is no generalized increase in met-enkephalin levels as a secondary response to hypoxia or as a primary event in the pathogenesis of this condition.

Overactivity of the endogenous opioid system may occur as a result of several mechanisms: supersensitivity of the receptors, increased number of receptors, defective degradation or inactivation of the peptides, abnormal "enkephalinase" activity,[44] or overproduction. In fact, even sudden death associated with exogenous opiate abuse may not necessarily be a dose-related phenomenon.[45,46] Current data suggest that drug "overdose" may often result from a failure of tolerance.[47] Thus, the lack of a pronounced difference in met-enkephalin levels between SIDS cases and controls does not decrease the likelihood of primary involvement of the endogenous opioid system in the pathogenesis of SIDS, since only overproduction and perhaps defective degradation of met-enkephalin were addressed by the present study.

Rhythmic respiratory drive is believed to be generated by various nuclei in the medulla.[48] Opiate receptors are found in these nuclei,[13] and enkephalins are present in the medulla.[25,28] For these reasons, the met-enkephalin content in the medullas of SIDS victims is of particular interest. In comparison with the variation found in the other brain areas, met-enkephalin content in the medullas of SIDS victims was within a narrow range (10.5 to 61 pmol/g); 9 of 14 cases (64%) had between 26 and 40 pmol/g (Table 3). Medulla met-enkephalin levels in the SIDS cases were not detectably different from those in the hypoxic control group. Both SIDS cases and the hypoxic controls had levels higher than the trauma controls. However, direct comparison with the traumatic control values is made difficult by the small number of cases (two), their older age (11 months and 12 months), and the observed inverse relationship with age. Although all the medulla values may be well within a normal range, it is curious that values in infants dying from a quiet, sudden death would have levels that are indistinguishable from those in infants dying of severe congenital

cyanotic heart disease with the stressful histories noted in Table 3, since the endogenous opioid system may be activated by stress.[49] Thus, it is possible that both the SIDS values and the chronically hypoxic control values are abnormally high, but more study is needed.

The levels of met-enkephalin and beta-endorphin in the lungs of SIDS victims are of great interest because of recent reports that enkephalin analogues stimulate pulmonary J-receptors in rats.*[50,51] The reflex produced by J-receptor stimulation causes a decrease in blood pressure, increased laryngeal resistance, and apnea, followed by tachypnea and bradycardia.[52-54] Specifically, Sapru and associates[50,51] demonstrated that enkephalin analogues injected into the right atrium of decerebrate rats stimulated pulmonary J-receptors and produced bradycardia and apnea within 1 to 2 seconds. Apnea was followed by rapid, shallow breathing. The duration of apnea was dose-related, could be blocked by pretreatment with naloxone, and was not observed with physiologic saline injection. Beta-endorphin caused these same effects.[55] Also noted was a naloxone-reversible increase in laryngeal and lung resistance.[56,57] Although these results should be interpreted with caution,[58] these reports are fascinating, and if they are confirmed by other groups they would provide an attractive mechanism for the pathogenesis of SIDS. In fact, the possibility that prolonged apnea in infancy may result from activation of pulmonary J-receptors has been previously noted.[59]

Plasma beta-endorphin levels show a clear diurnal variation in adults, with a two-fold difference between peak and trough levels.[60] The peak plasma values of beta-endorphin in adults occur between 4 a.m. and 10 a.m. This is the same time of day that SIDS is most commonly noted to occur.[9,61] Whether such a diurnal variation in plasma values occurs in infancy is unknown, but it is tempting to speculate that the apnea noted in normal infants, the increased duration and frequency of apnea in infants who have had a near miss for SIDS, and the terminal apnea in SIDS victims may all be due to differing degrees of stimulation of pulmonary J-receptors by early morning peaks of plasma beta-endorphins. This would explain the circadian distribution of apnea (peak between 1 a.m. and 6 a.m.) which has been noted during 24-hour monitoring in infants.[7]

Such a mechanism would also account for the case reports of sudden death in infants with congenital adrenal hypoplasia.[62,63] These infants would be expected to have high levels of ACTH (in the one case in which it was measured it was elevated),[62] which, studies suggest, would be accompanied by elevated plasma levels of beta-endorphin.[64,65]

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*There are three types of pulmonary receptors (vagal afferents): stretch, irritant, and unmyelinated J-receptors. Although there are some species variations, the general characteristics of these receptors are similar for man, cat, rabbit, and dog.[52,53]

Very small (femtomolar range) but measurable amounts of methionine-enkephalin and beta-endorphin were found in lung tissue from SIDS and control infants (Table 4). We expected that measurable levels of these peptides would be present in human lung tissue because of earlier reports of enkephalins[66] and opiate receptors[67] in mammalian lungs, and from the immunohistochemical demonstration of leu-enkephalin in endocrine cells of lung tissue from human fetuses, newborns, children, and adults.[68]

The values of beta-endorphin which we found in lung tissue (315 to 639 fmol/g wet weight of tissue) are considerably higher than values reported in human plasma (adults, 4 fmol/ml plasma;[60,65] cord blood, approximately 16 fmol/ml plasma;[69,70] term amniotic fluid, approximately 20 fmol/ml[70]). The levels of met-enkephalin in 1 g wet weight of lung tissue ranged from 520 to 11,000 fmol, which is very low in comparison with levels in brain tissue and is similar to values found in 1 ml of plasma (approximately 100 fmol of met-enkephalin per milliliter of plasma).[71] There was no difference between SIDS and control infants with regard to the levels of met-enkephalin or beta-endorphin in lung tissue (Table 4). This finding suggests that if pulmonary J-receptor activation is involved in the pathogenesis of SIDS, postmortem evidence for such involvement cannot be derived from radioimmunoassay of lung tissue extracts by means of the method described. Plasma levels of beta-endorphin in near-SIDS infants or postmortem plasma levels of beta-endorphin in SIDS victims may be more revealing.

It is interesting to note the similarities in pulmonary pathology found in SIDS victims and in cases of sudden death due to opiate abuse--pulmonary edema is common in both instances.[72,73] Frothy hemorrhagic fluid in the tracheobronchial tree and around the mouth and nose has been reported in 56% of SIDS cases.[72,74] Frothy fluid around the mouth is also commonly found among addicts who have experienced sudden death due to opiate injection.[45,73] Petechial hemorrhages under the pleura have been noted in SIDS infants[75] and also among heroin addicts who have died suddenly.[76] Although these similar pathologic findings may result from a completely different pathophysiology, or from similar pathophysiology but different etiology, the similarities in pathology are interesting in light of our hypothesis and have not been previously noted.

The vulnerability of the infant to SIDS between the ages of two months and four months may be related to a postnatal ontogenic development of the endogenous opioid system. Several studies suggest that there is a postnatal ontogenic development of the endogenous opioid system in rats.[77-79] Administration of morphine or naloxone to female rats before and during pregnancy has been shown to alter significantly the normal postnatal regional pattern of development of [^{3}H]met-enkephalin binding sites in the brains of offspring.[79] If SIDS was caused by an abnormality of the endogenous opioid system, then these studies, which suggest that maternal opiate abuse perturbs the normal ontogeny of the

endogenous opioid system in offspring, would explain the strikingly increased incidence of SIDS among infants of mothers addicted to opiates or on methadone maintenance.[80-82]

CONCLUSIONS

Primary involvement of the endogenous opioid system in the pathogenesis of SIDS provides an attractive mechanism for silent, sudden, infant death. Careful consideration of our present knowledge of SIDS and the endogenous opioid system yields a hypothesis that coherently explains all of the unique features of SIDS. We assayed various tissues for met-enkephalin and lung tissue for beta-endorphin. No pronounced differences in met-enkephalin levels between SIDS and control infants were apparent. However, further study of the rate of change in medullary met-enkephalin concentration with respect to age is indicated.

Given the inherent difficulties associated with postmortem analysis and the attendant problems of data interpretation, a clinical study using naloxone in near-miss infants may be the most definitive way of assessing the role of endogenous opiates in SIDS and near-SIDS.

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