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Effect of Protein Malnutrition on Morphine Analgesia and Brain Serotonin Profile of Adult Rats

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In the present study, malnutrition was induced in adult rats by feeding a protein deficient (7% protein) diet for 36 d in order to study its effect on morphine analgesia and on brain serotonergic activity. In the early phase of malnutrition, both, morphine analgesia and serotonergic neuronal activity were increased concomitantly when compared to that of their respective control values from nourished adult rats. On the contrary, both morphine analgesia and serotonergic neuronal activity in brain were decreased in the late phase of protein deprivation. These results indicate that morphine analgesia in malnourished adult rats is primarily governed by serotonergic neuronal activity of the brain. In contrast to above, malnourished adult rats also showed an exposure dependent progressive decrease in the analgesic effect of morphine while malnourished rats rehabilitated on protein-rich diet for a period of two weeks did not show such anomaly. The decrease in morphine analgesia on its repeated exposure in malnourished adult rats may be related to the decrease in formation of N-normorphine, an active metabolite of morphine responsible for its analgesic effect, due to decrease in microsomal mixed function oxidase activity in the brain and liver.

Studies conducted in a number of laboratories have suggested that surgical and pharmacological manipulations that modify brain serotonin concentrations are also associated with the changes in the response to painful stimuli[1] and consequently may alter the analgesic potency of morphine and other narcotics[1-3]. Dietary protein deficiency, including starvation, is known to alter brain serotonergic neuronal activity in animals[4-6]. The present study was, therefore, undertaken to examine the effect of protein deficient diet on the analgesic response of morphine and on brain serotonergic neuronal activity in order to find out whether analgesic effect of morphine in undernourished brain is solely modulated through serotonergic neuronal activity or some other mechanism(s) also contribute in addition to its well documented action through opiate receptors.

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MATERIALS AND METHODS

Adult Fisher rats (100-125 g, 6-7 w) were fed protein-rich diet (Lipton India Ltd.) in powdered form and water *ad libitum* for 2 w in order to acclimatize them to environmental conditions of the departmental animal house (14 h light, 10 h darkness, room temp. 23-25°). The animal experimental protocols have been approved by the Institutional Animals Ethics Committee.

Induction of malnutrition in adult rats:

Malnutrition in adult rats was induced according to the method of Stern *et al.*[7]. Control rats were fed unrestricted protein-rich diet (21% protein) in the powdered form. Malnourished rats were fed unrestricted but protein deficient diet made by mixing 1 part of protein-rich diet with 2 parts of sago powder, containing predominantly starch (i.e. 7% protein) for 36 d. On d 37, malnourished rats were rehabilitated by feeding protein-rich diet for a period of 2 w.

Estimation of brain serotonin and rate of accumulation:

Brain serotonin level was estimated by the method of Curzon and Green[8]. Whole brain (excluding cerebellum) was used for estimation of brain serotonin level. For determining the rate of accumulation of brain serotonin, the method of Neff and Tozer[9] was used. Pargyline hydrochloride (Sigma) was administered in doses of 75 mg/kg (i.p.) in both control and malnourished rats (on d 18 and 36 of malnourishment). Brain samples were dissected out at 0, 30 and 60 min intervals after pargyline injection. Brain serotonin level was estimated as mentioned above.

The rate of accumulation of brain serotonin was calculated by subtracting the mean 0 h brain serotonin value from individual brain serotonin value obtained after 30 and 60 min of pargyline administration in the same group and finally converting it to per h expression by a suitable multiplication. The mean value of rate of accumulation of brain serotonin was expressed as ng/g/h of fresh brain tissue.

Morphine analgesia:

Analgesic effect of morphine hydrochloride (4 mg/kg; Government Opium Factory, Ghazipur) was studied in nourished controls, in rats fed a protein deficient diet for 4, 9, 18, 29 and 36 d and in rehabilitated malnourished rats fed protein-rich diet for 2 w (i.e. on d 50 of experiment), by the rat-tail-hot wire technique[10] using a Techno analgesiometer (Techno Pvt. Ltd., Mumbai).

Statistical analysis:

The results were analyzed by Student's 't' test after calculating the mean values and the standard error of the mean for the duration of morphine analgesia and brain serotonin levels between malnourished/rehabilitated and nourished groups. Statistical significance was evaluated at a probability level of $P<0.05$.

RESULTS

Effect of protein deficient diet on brain serotonin profile of adult rats:

Concentration of serotonin in brain of adult control rats was 894±13.8 ng/g of fresh tissue. Malnourished rats initially demonstrated a progressive increase in brain serotonin levels up to d 18 (1033±59.9 ng/g of fresh tissue, $P<0.05$) followed by a gradual decrease thereafter. On d 36, brain serotonin levels of malnourished rats were comparatively but not significantly lower (838±35.9 ng/g of fresh tissue) than that of control rats as shown in Table 1.

TABLE 1: EFFECT OF PROTEIN DEFICIENT DIET ON BRAIN SEROTONIN CONCENTRATION OF ADULT RATS

Days after PD diet	n	Serotonin concentration$ (ng/g)
C	12	894±13.8
PD		
9	5	953±18.8*
18	5	1033±59.9*
29	5	1003±51.0
36	5	838±35.9

C stands for control adult rats, PD means adult rats maintained on a protein-deficient diet. $ represents Mean±SEM. Concentration of serotonin in whole brain excluding cerebellum. *Significantly different from controls at $P<0.05$ and n is the number of animals used.

Effect of protein deficient diet on rate of accumulation of brain serotonin in adult rats:

The mean value for the rate of accumulation of brain serotonin in control rats after pargyline (75 mg/kg, i.p.) was 213±13.4 ng/g/h of fresh tissue. In protein-deprived rats,

TABLE 2: EFFECT OF PROTEIN DEFICIENT DIET ON THE RATE OF ACCUMULATION OF BRAIN SEROTONIN OF ADULT RATS

Days after PD diet	n	Rate of accumulation of serotonin$ (ng/g/h)
C	12	213±13.4
PD		
18	12	297±29.8*
36	10	189±26.9

C stands for control adult rats, PD means adult rats maintained on a protein-deficient diet. $ represents mean±SEM of the rate of accumulation of serotonin concentration in whole brain excluding cerebellum. *Significantly different from controls at $P<0.05$ and n is the number of animals used.

there was a significant increase in the rate of accumulation of brain serotonin on d 18 (297±29.8 ng/g/h of fresh tissue) but further continuation of protein deficient diet for 36 d had an opposite effect (189±26.9 ng/g/h of fresh tissue, Table 2).

Effect of protein deficient diet on morphine analgesia:

The duration of latency of tail flick response in control adult rats to applied radiant heat stimulus (6 mA) was 5.0±1.0 s. Morphine hydrochloride (4 mg/kg, s.c.) had increased the duration of latency to tail flick response to 7.3±0.67s above its control value (Table 3). Adult rats maintained on a protein deficient diet demonstrated a progressive increase, but not before d 4, in the duration of latency to tail flick response to morphine. The maximum increase in the duration of latency to tail flick response (13.4±1.49 s) was observed on d 18 of malnourishment (Table 3). Afterwards, its analgesic effect in malnourished rats started to decline. On d 29 and thereafter, the increase in the duration of latency to tail flick response in protein deficient rats to morphine was similar to that of control rats (Table 3).

TABLE 3: EFFECT OF PROTEIN DEFICIENT DIET ON MORPHINE (4 MG/KG, S.C.) ANALGESIA

Days after PD diet	n	Increase in latency to tail flick response$ (in s) after morphine
C	31	7.31±0.67
PD		
4	10	7.31±1.22
9	18	12.1±1.09*
18	15	13.4±1.49*
29	12	8.78±1.59
36	12	8.63±0.72

C stands for control adult rats, PD means adult rats maintained on a protein-deficient diet. $ represents mean±SEM. * Significantly different from controls ($P<0.05$) and n is the number of animals used.

Effect of morphine on its repeated exposure in malnourished and subsequently rehabilitated adult rats:

Table 4 shows that repeated administration of morphine (4 mg/kg, s.c.) did not exhibit any change in its analgesic response, when measured in terms of increase in duration of latency to tail flick response in control adult rats but its analgesic effect decreased considerably in adult rats maintained on a protein deficient diet. The decrease in analgesic effect of morphine was exposure dependent and was significant only after its third exposure when compared to that of the first one (Table 4). On the other hand, malnourished rats rehabilitated on protein-rich diet for 2 w did not exhibit such an anomaly (Table 4).

DISCUSSION

In the present study, adult rats fed a protein deficient (7% protein) diet for 36 d showed an increase in serotonergic neuronal activity in the early phase (evident by an increase in serotonin level as well as an increase in the rate of accumulation of serotonin in brain on d 18) and a decrease in serotonergic neuronal activity in the late phase (evident by a decrease in serotonin level as well as a decrease in the rate of accumulation of serotonin in brain on d 36) of protein deprivation (Tables 1 and 2). The cause of early rise in brain serotonin biosynthesis in adult rats maintained on a high carbohydrate diet is due to an increase in brain tryptophan level through the insulin release[4,11]. The later enhances the cellular uptake of tryptophan by increasing neuronal cells and blood brain barrier permeability[12]. On the other hand, the decrease in serotonin biosynthesis in brain in the late phase (i.e. after 5 w) of malnourishment could be explained on the basis that tryptophan is an indispensable amino acid, which cannot be synthesized *de novo*, hence, all tryptophan molecules available for serotonin biosynthesis in brain and in plasma must be derived from the diet. Ingestion of a diet deficient in protein, particularly in tryptophan, for a considerable period of time may result in the exhaustion of its reserve pool available for serotonin biosynthesis in brain and in plasma[4,6,13].

In the present study, the duration of morphine analgesia varied considerably on different d of protein deprivation. Earlier, many workers have reported that analgesic effect of morphine is serotonergic neurotransmitter mediated[2,3,14,15]. It was, therefore, conceived that any changes observed in the duration of morphine analgesia may also be associated to the changes observed in the serotonergic neuronal activity of brain during malnourishment. In the present study, altered analgesic response of morphine in adult rats observed on different d of malnourishment (Table 3) was more or less similar to the changes observed in steady state level and rate of turnover of serotonin biosynthesis (Tables 1 and 2) in malnourished brain i.e. an increase in the analgesic effect of morphine was associated

TABLE 4: MORPHINE ANALGESIA ON REPEATED EXPOSURE OF MORPHINE IN CONTROL, PROTEIN-DEFICIENT AND NUTRITIONALLY REHABILITATED ADULT RATS

No of exposures	Increase in latency to tail flick response$ (s) after morphine				
	n	C	Days after PD diet	n	PD
I	13	7.52±1.12	9	18	12.1±1.09
A,II	9	7.04±1.12		10	9.2±0.87*
I	9	7.08±1.45	18	15	13.4±1.49
B,II	7	8.04±0.91		7	11.7±1.16
A,B,III	7	6.66±0.72		8	8.7± 0.50*
I	10	7.21±1.22	29	12	8.8± 1.59
B,II	7	7.53±1.24		9	9.2±1.30
B,C,III	6	7.72±2.03		6	4.7±0.53*
I	8	7.42±1.20	36	12	8.6±0.72
C,II	8	7.34±1.02		12	6.8±0.53*
B,D,III	4	7.65±1.61		7	3.9±0.63*
C,E,III	6	7.75±2.07	50@	12	6.8±1.16
B,D,E,IV	4	6.58±1.86		7	7.2±1.72

C stands for control adult rats, PD denotes adult rats maintained on a protein deficient diet, @ denotes protein deficient diet was replaced by protein-rich diet on day 37 of the experiment. Rats exposed to morphine: A on d 4; B on d 9; A,B on d 4 and 9; B,C on d 29 and 36; B,D,E on d 9, 18 and 36. I, II, III and IV indicates the actual number of exposure of morphine on a particular day, from the first day of feeding of protein-deficient diet. $ represents mean±SEM. *Significantly different from morphine analgesia observed on first exposure ($P<0.05$) and n is the number of animal used.

with an increase in brain serotonin biosynthesis in malnourished rats (i.e. on d 18 of malnourishment) as compared to that of control nourished rats and vice-versa (Tables 1, 2 and 3).

On the contrary, malnourished, but not control adult rats showed an exposure dependent decrease in analgesic response of morphine which was quite significant on its third exposure (Table 4) while malnourished rats rehabilitated on protein-rich diet for 2 w did not exhibit such an anomaly (Table 4). Many workers have reported that repeated administration of smaller doses of morphine (4-8 mg/kg) did not have any effect on brain serotonin concentration[16-18]. It was, therefore, presumed that morphine in the doses used in the present study (i.e. 4 mg/kg) did not contribute any influence on brain serotonin biosynthesis. Chronic administration of morphine in adult rats[19] as well as adult rats maintained on protein deficient diet for 15 d or more, concomitantly, demonstrated a decrease in liver microsomal MFO (mixed function oxidases) activity[20-22] which could be partially reversed if protein deficient diet is replaced by a diet containing adequate protein[22]. The depressed liver microsomal MFO activity on repeated administration of morphine was considered to be a proposed mechanism of tolerance to morphine response[23,24] because they postulated that N-demethylated metabolite of morphine (nor morphine), but not morphine, is essential for its analgesic effect. The present schedule of repeated administra-

tion of morphine dose (*i.e.* 4 mg/kg, s. c.) might be sufficient to depress the microsomal MFO activity of both brain and liver of malnourished, but not nourished, adult rats to N-demethylate morphine in which microsomal MFO activity had already been suppressed by feeding a diet deficient in protein. Thus, decrease in analgesic response of morphine in malnourished adult rats might be attributed to the less availability of nor-morphine in brain from administered dose of morphine as compared to that of nourished adult rats in which microsomal MFO activity was not significantly depressed. The role of brain serotonergic neuronal activity in modulation of morphine analgesia might be involved in the later stages after the formation of normorphine compound.

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