operated animals after its injection, are indicative of pronounced disturbances in gas exchange and energy metabolism.

It follows, then, that inactivation of carotid receptors leads to functional manifestations of prediabetes, and that the diabetogenic action of streptozotocin in glomectomized animals is accompanied by greater alterations in blood levels of glucose and hemoglobin and in red cell and gas exchange indexes than in sham-operated animals. This study has thus demonstrated an important physiological role of the carotid sinus reflexogenic zones in the development of adaptive responses in health and in diabetes mellitus.

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Comparative Analysis of Effects from Prolonged Peripheral and Intracerebral Exposure to β-Endorphin

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During a long-term (7-day) continuous exposure of rats to β-endorphin from an implanted minipump by the subcutaneous route, changes in their motor activity (stereotypy) and pain sensitivity (hypoalgesia) were similar, though less marked, than those observed during such exposure by the intracerebral route, whereas the change in feeding behavior with the subcutaneous (peripheral) route of β-endorphin administration (hyperphagia) was opposite to that seen with intracerebral administration (hypophagia).

Key Words: *β-endorphin; osmotic minipumps; feeding behavior; pain sensitivity*

It has been demonstrated in numerous experimental studies that most endogenous regulatory peptides (RPs) are formed through specific proteolytic processing from high-molecular precursor proteins. The precursor protein for β-endorphin (En), β-lipotropin, and adrenocorticotropic hormone is proopiomelanocortin [3,6].

There is biochemical evidence that mammals possess enzyme systems responsible for the biotransformation, inactivation, and elimination of peptides [6,13]. As has frequently been pointed out [1,13], this explains why it is difficult, in experiments using exogenously administered RPs, to evaluate the real role of peptides in the regulation of physiological functions, especially since the experimentally used doses of RPs far exceed their concentrations in tissues and biological fluids. The levels of En and many other RPs in the body are regulated by highly complex feedback mechanisms. The major components of the regulatory system concerned are the hypothalamus, pituitary, and adrenals [3,6]. It has been shown that the level of En in the anterior pituitary correlates most closely with its plasma level [3,7], that the main source of En contained in the

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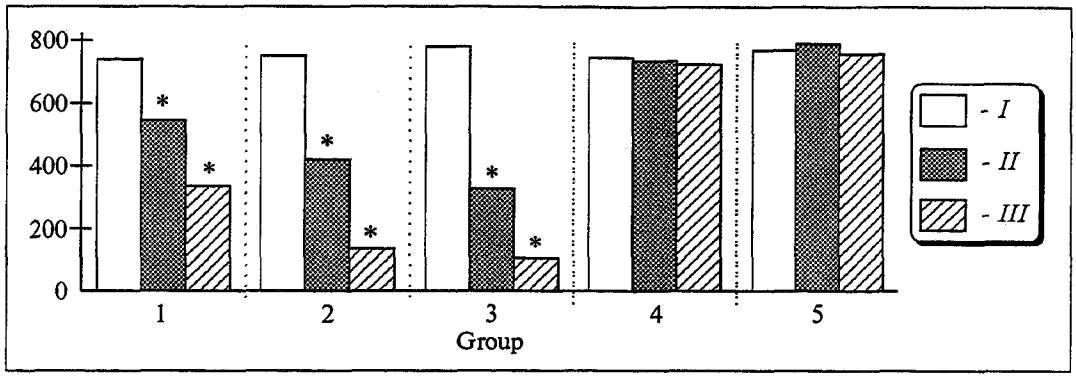

Fig. 1. Total motor activity of rats at different times. Ordinate: number of motor acts (horizontal and vertical activities) over 5 min. Here and in Figs. 2 and 3: *I*) before minipump implantation; *II* and *III*) on days 3 and 7 postimplantation. *$p<0.05$ in comparison with the preimplantation period.

cerebrospinal fluid is most likely the hypothalamus, and that variations in the concentration of this peptide reflect processes of its intracellular metabolism and secretion [3,5].

In view of the foregoing, we decided to perform a study on rats, to see how long-lasting peripheral or intracerebral administration of En in microdoses would influence the behavior of the animals.

MATERIALS AND METHODS

β-Endorphin was administered from osmotic minipumps (Alzet), Model 2001, of esterified cellulose enclosed in a small capsule fitted with a regulator of the rate at which the solution contained in the capsule enters the external medium. The total capacity of a minipump is 233±9 μl and the standard discharge rate of osmotically active substances dissolved in physiological saline is 1.1 μl/h at temperatures of 4 to 42°C and pressures of 0.5 to 25 atmospheres. With osmotic minipumps of this model implanted under the skin in the suprascapular region, the substance of interest such as En can be continuously administered for 7 days. For intracerebral administration of En, the minipump outlet was connected via a polypropylene catheter to a cannula implanted into a lateral ventricle of the brain.

In the first experimental series, the time course of En discharge from osmotic minipumps incubated in physiological saline (1 ml) at 38°C was followed *in vitro*. During the incubation period, samples were taken from the solution filling each minipump (200 μl of saline containing 75 or 150 μg of En - these two doses were later used in behavioral experiments) - and En levels in the samples were determined with radioimmunoassay and a modification [2] of the dot-blotting technique described by Hawkes *et al.* [9].

The second experimental series was carried out on 32 test and 16 control male Wistar rats. Of the 32 test rats, 22 were administered En continuously from the subcutaneously implanted pump over a period of 7 days in a total dose of 75 μg (group 1, n=12) or 150 μg (group 2, n=10). The remaining 10 test rats (group 3) received En, as described above, in a lateral brain ventricle in a total dose of 15 μg over the same period. Of the 16 control rats, which were implanted with an osmotic minipump containing physiological saline, 10 received this solution subcutaneously (group 4) and 6 intracere-

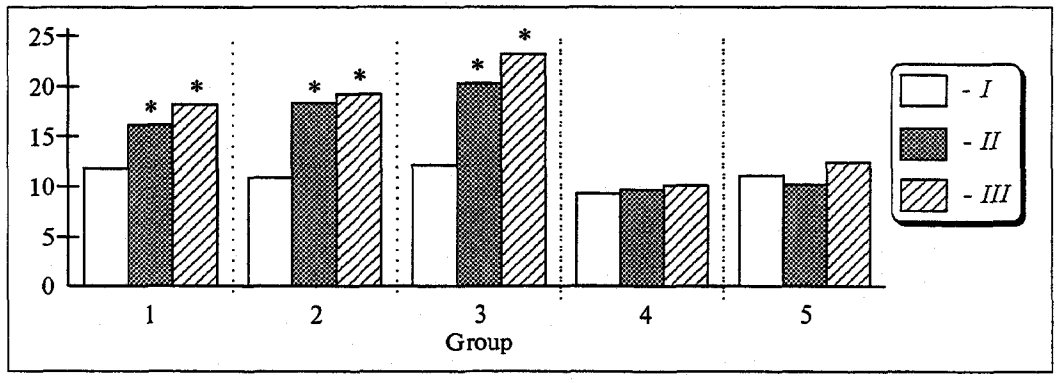

Fig. 2. Pain sensitivity thresholds (as estimated by the tail–flick response) in rats at different times. Ordinate: latency (sec) of the tail–flick response.

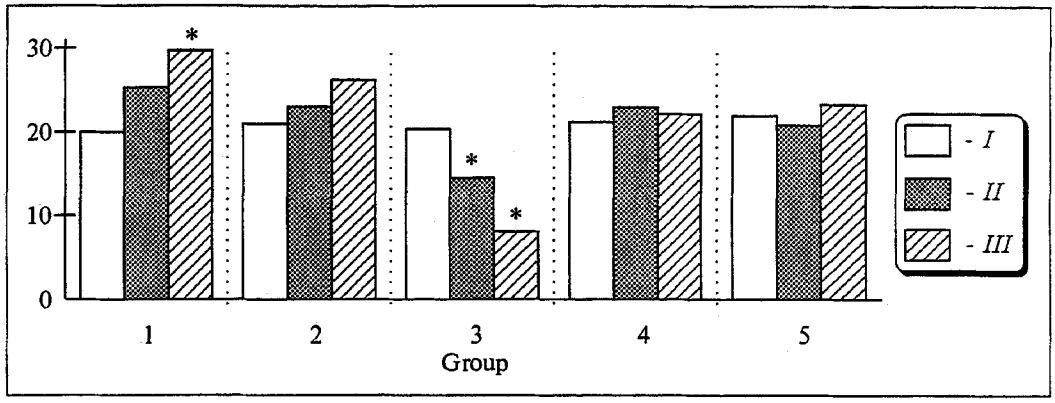

Fig. 3. Food consumption by rats at different times. Ordinate: daily food intake, g.

brally (group 5). The minipumps and cannulas were implanted under light ether anesthesia.

For each rat, daily food and water intake and body weight were measured. Before and on days 3 and 7 after implantation of the minipump, the motor activity of the animals was recorded with a Varimex instrument (Columbus Instruments) and their pain sensitivity thresholds were estimated by recording the latency of the tail-flick response.

The results were statistically analyzed by Student's t test.

RESULTS

As shown by the immunoblotting and radioimmunoassays in the first experimental series, the 7-day incubation (at 38°C) of osmotic minipumps containing 75 or 150 μg of En in physiological saline did not lead to degradation of the peptide. Its biological activity in samples of the solution filling the minipumps was retained; thus, the introduction of 5 μl of this solution into a brain ventricle of intact rats raised their pain sensitivity thresholds significantly by an average of 40% ($p<0.05$). The rates of En discharge from the minipumps containing 150 and 75 μg of the peptide were 19.8 and 9.9 μg/day, respectively.

The behavioral tests of rats administered En subcutaneously from a minipump showed a progressive decline of motor activity during the 7-day period ($p<0.05$), particularly in animals given the higher (150 μg) dose (Fig. 1), and an increasing incidence of motor stereotypy.

With the subcutaneous route of En administration, pain sensitivity thresholds, particularly in rats given the higher dose, were significantly higher ($p<0.05$) than in the control animals (Fig. 2).

Prolonged exposure to En by the intracerebral route reduced pain sensitivity to a greater extent than by the subcutaneous (Figs. 1 and 2), and produced more clearly defined motor disturbances in the form of intensive stereotypy.

The changes in pain sensitivity and motor activity observed in this study were similar to those recorded after a single peripheral or intracerebral administration of exogenous En [10,12,14], but they persisted for a long time and were significant.

Comparison of food consumption in the groups administered En under the skin (75 and 150 μg over 7 days, delivered at rates of 6.6 and 13.2 ng/min, respectively) and into the brain ventricle (15 μg, delivered at a rate of 1.3 ng/min) showed that the rats receiving En peripherally consumed more food per day ($p<0.05$), whereas those receiving it by the central route consumed less ($p<0.05$), as compared to the baseline period preceding implantation of the minipump (Fig. 3); in contrast, no differences from the baseline were observed in water consumption or body weight.

These findings are consistent with what is known about the role En plays in the regulation of feeding behavior. For example, the concentration of this peptide decreases in the hypothalamus and increases in the anterior pituitary and cerebrospinal fluid of animals deprived of food for 1-3 days [3,8], and its levels in the hypothalamic structures involved in shaping feeding behavior have been shown to correlate closely with the rhythm of feeding activity in rats [3,11]. A rise of En in the hypothalamus is believed to be a sign of satiety [4,8,11]. Elevated plasma levels of this peptide were recorded after insulin injection, during spontaneous food intake at nighttime, and after food deprivation [7,8,12]. En was found to increase food consumption by rats in low doses (0.01-1.0 nM) and to decrease it substantially in higher doses [3,12].

In the present study, the long-lasting continuous exposure to En produced a symptom complex of behavioral effects including alterations in pain sensitivity, motor activity, and feeding behavior. It should be noted that whereas the peripheral and intracerebral En administrations had similar effects

of pain sensitivity and motor activity, they affected food intake in opposite ways.

According to current concepts, the broad spectrum of biological activity observed for most RPs is due to the fact that the exogenous administration of such peptides results in their direct interaction with specific cellular receptors, in stimulated or inhibited release of other physiologically active substances (RPs, transmitters, etc.), and in their interaction with enzymes that synthesize or degrade endogenous RPs. During the latter interaction, the exogenous peptides compete with endogenous peptides for receptor binding sites and with endopeptidases, thereby suppressing the degradation of endogenous RPs [6,13].

The foregoing suggests that En continuously delivered from an osmotic pump for a prolonged period causes compensatory changes in the synthesis, secretion, and degradation of endogenous En and in the sensitivity of central and peripheral opiate receptors, and that these changes are manifested at the organismic level in the behavioral changes described above.

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