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Dose-Related Potent Brain Stimulation by the Neuropeptide Endothelin-1 After Intraventricular Administration in Conscious Rats

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CHEW, B. H., D. F. WEAVER AND P. M. GROSS. *Dose-related potent brain stimulation by the neuropeptide endothelin-1 after intraventricular administration in conscious rats.* PHARMACOL BIOCHEM BEHAV **51**(1) 37–47, 1995.– Injection of the neuropeptide, endothelin-1 (ET, range of 3–9 pmol), into a lateral ventricle (ICV) of rats produced barrel rolling and other convulsions including ataxia, forelimb and facial clonus, nystagmus, and tonic extension of the tail and hindlimbs. Using the quantitative autoradiographic [^{14}C]deoxyglucose method, we resolved the focal hypermetabolic correlates of the convulsive activity in numerous brain regions. The present study tested whether the effects of ET were dose dependent by assessing 13 behavioral, 9 physiological, and brain metabolic responses in six individual structures of rats treated separately with ICV ET in doses between 1.5 and 18 pmol. Barrel-rolling convulsions, having a threshold for onset at 3 pmol, displayed increased incidence and severity, and a shorter latency to onset, with the higher ET doses. Within 10–20 min, ET evoked dose-dependent increases in mean arterial pressure and plasma glucose levels, and a significant reduction in arterial PCO_2. Among brain structures, the periventricular caudate nucleus near the injection site had an elevated rate of glucose metabolism (+60%) at a 3 pmol threshold. The substantia nigra pars reticulata, medial terminal nucleus of the accessory optic tract, rostral lamella of the inferior olivary nucleus, cerebellar paramedian lobule, and cerebellar copula pyramis, all of which have moderate to dense populations of ET-1 receptors and are related by anatomical connections, displayed significant metabolic stimulation by 9 pmol ET (+47 to +122%). The behavioral, physiological, and focal hypermetabolic effects of the central ET appear to be time coordinated, interrelated, and dose dependent. Identification of the threshold dose for central actions of ET at 3 pmol ICV reveals this peptide as the most potent neuroactive substance yet described in vivo.

Barrel rotation Proprioception Eye control Cerebellum Metabolic mapping Epilepsy model

ENDOTHELIN-1 (ET), a 21-amino acid peptide first thought to be mainly a vascular autacoid (47), has numerous roles in normal and pathologic functions of several organ systems (27,30,37,48). When injected into a lateral cerebral ventricle of conscious rats, ET provokes convulsions characterized by nystagmus, facial and forelimb clonus, ataxia, and a rotational phenomenon termed barrel rolling (12,26,32). These convulsions are accompanied by an increase in mean arterial pressure, decrease in arterial PCO_2, and elevation of plasma glucose concentrations, responses that are likely the result of systemic sympathetic activation (15,28,36,46).

Throughout the brain, ET displays distinct binding densities, immunoreactivity, and messenger RNA (24,40). Its receptor populations are particularly dense among nuclei of the oculomotor and vestibular systems, and in the Purkinje and granule cell layers of the cerebellar cortex (20,21,24,35). Because the binding pattern for ET is nonvascular, the activity of this peptide is probably neuronal as well as vascular. Therefore, ET may be a neuromodulator substance or neuronal signaling molecule (9,10,16,24,40). Consistent with this hypothesis, the quantitative autoradiographic [^{14}C]deoxyglucose method applied to rats given an intracerebroventricular (ICV)

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injection of ET revealed specific hypermetabolic responses in periventricular structures, eye and motor control nuclei, and the cerebellar cortex (6,12,13,15,18). The stimulation of anatomically connected brain structures and specific subregions indicates that complex neural circuits were engaged during the convulsive activity evoked by this peptide. Because ICV injection of other neuropeptides, including arginine-vasopressin (22,25,43-45) and somatostatin (1,4,5,8), causes similar convulsions, a reasonable working hypothesis is that neuropeptides such as ET are important for modulating central visuovestibular circuits controlling proprioception.

In early studies on the central effects of ET, Ouchi and co-workers (36) found that the arterial blood pressure and plasma catecholamine levels were increased in a dose-dependent manner by escalating doses of ICV ET (8.25-66 pmol). Their highest doses ($\geq$ 33 pmol), however, caused death from pulmonary edema within 5 to 20 min after injection (36). To date, the cerebral hypermetabolic effects of subpathological doses of ICV ET have not been well correlated in an integrated study with the behavioral and physiological effects resulting from ventricular injection of this peptide. Based on the doses employed by Moser and Pelton (32) and Ouchi et al. (36) and our previous studies with 9 pmol ICV injections of ET (6,12-19), we determined in the present work if the effects of centrally administered ET were associated with dose by evaluating three parameters presumed to be interrelated: a) incidence and severity of the behavioral symptoms, b) the changes in cardiovascular and blood chemical status of the animal, and c) alterations in local cerebral glucose utilization of selected nuclei by application of the quantitative autoradiographic [^{14}C]deoxyglucose technique.

METHOD

Rat Preparation and Experimental Conditions

Fifty-nine adult male Sprague-Dawley rats (246-457 g) were anesthetized with 65 mg/kg IP sodium pentobarbital, positioned in a stereotaxic frame with the skull leveled, and implanted with lateral ventricular cannulae. Using bregma as a reference, we trephined a hole in the left parietal bone to expose the dural surface at coordinates +1.8 mm L and −1.0 P. A 22 gauge, stainless steel guide cannula was lowered 3.7 mm ventral from the dural surface into the left lateral cerebral ventricle at the level of the hippocampal fimbria and caudate nucleus. We placed three jeweler's screws in the skull to anchor a cranioplastic cap holding the cannula through which we inserted a 28 gauge obturator with a nylon screw fitting to maintain patency (Plastic Products Co., Roanoke, VA). The rats were placed in individual cages with food and water ad lib and allowed at least 48 h for recovery.

The rats were fasted 18 h prior to the experiment. While the rats were anesthetized with a mixture of 1.5% halothane, and 1 : 1 nitrous oxide and oxygen, femoral venous and arterial catheters were inserted. We gave the rats 500 U of heparin IV for anticoagulation and placed loose-fitting plaster casts around the hindquarters for gentle restraint of the animals on lead blocks; this is a common procedure developed in the procedures of the [^{14}C]deoxyglucose method for assessment of brain glucose metabolism in conscious rats (38). Use of the hindquarters cast on the rats was necessary to permit the sampling of arterial blood and measurement of arterial pressure. Although this procedure limited the full expression of ET-induced barrel rolling, our experience with unrestrained animals indicated that barrel rolling could be reliably assessed by the rat's cephalad motor response, which included twisting of the upper torso under the long axis of the body. Furthermore, lower body restraint permitted more precise observation of other types of convulsive activity, such as nystagmus and facial clonus. Body temperature was regulated by a biofeedback heat lamp connected to a rectal thermometer.

Two hours were allowed for recovery from the anesthetic, at which time the appropriate ICV injection was given: saline (1.5-3 μl, n = 12) or ET (Peptides International, Louisville, KY) in the following doses—1.5 pmol (n = 5), 3 pmol (n = 10), 6 pmol (n = 14), 9 pmol (n = 13), or 18 pmol (n = 5) dissolved in 1.5 μl saline delivered over 75 s (0.25 μl/15 s) using a Hamilton syringe. Each rat was used in only one experiment and received only one dose of ET. A small amount of Evans blue dye was added to all solutions to confirm the accuracy of the injection inside the ventricle at postmortem. All ET doses (saline, 1.5, 3, 6, 9, and 18 pmol) were tested in the behavioral and physiological series, whereas only the following doses were used in the deoxyglucose experiments: saline, 3, 6, and 9 pmol ET. The 18 pmol injection could not be used for complete deoxyglucose experiments because it proved to be lethal between 10 and 20 min after injection.

Behavior and Physiology

Behavioral and physiological measurements were recorded before injection (control), 5, 10, 20, 30, 45, and 60 min following injection. Behavioral assessments were based on a checklist of 13 parameters, including barrel-rolling latency and incidence, and the degrees of such observer-graded responses as nystagmus, exophthalmos, forepaw dystonia, facial and forelimb clonus, tail extension, sniffing, and piloerection on a scale of 0 (absent), 1 (mild), 2 (moderate), and 3 (severe). Physiological determinations included continuous monitoring of arterial pressure (Gould Recorder 2200S), and arterial blood was sampled before and during the experiment for blood gas (Nova Stat Profile 2 Analyzer) and glucose analysis (Beckman Glucose Analyzer 2). A table of nine physiological determinations was completed for each rat.

Cerebral Glucose Metabolism

Whereas 36 rats were included in experiments recording only behavioral and physiological measurements, 23 others were used primarily to assess cerebral rates of glucose utilization using the quantitative autoradiographic technique employing [^{14}C]deoxyglucose as the tracer (38) (2-deoxy-D-[1-^{14}C]glucose, specific activity 50-60 mCi, mmol; American Radiolabeled Chemicals, St. Louis, MO). Briefly, [^{14}C]deoxyglucose was injected as a bolus dose IV (125 μCi/kg in 0.5 ml saline), after which 16 timed arterial blood samples were drawn at predetermined intervals to derive plasma concentrations of ^{14}C and glucose. After a 45-min circulation time, the rats were sacrificed by IV overdose with sodium pentobarbital and the brains rapidly extracted and frozen in isopentane at −40°C. The brains were sectioned at −20°C in a cryostat and cut in 20 μm-thick coronal sections collected on glass coverslips. Sections were taken in triplicate, and the coverslips were glued to Bainbridge mat boards and placed with [^{14}C]methylmethacrylate standards in cassettes for 14 days with OM-1 Kodak film (Rochester, NY). A fourth section from each group was taken for histological staining with thionin at 60°C to aid in identification of nuclei using light microscopy and the neuroanatomical atlas of Swanson (39).

We used a microcomputer-based imaging system (MCID, Imaging Research Inc., St. Catharines, Canada) to digitize and enhance the histological sections and corresponding auto-

radiographic images, sample optical densities within individual regions, and compute rates of tissue glucose metabolism according to the operational equation of the [^{14}C]deoxyglucose method (38).

Although an extensive metabolic analysis in the rat brain is possible by application of the autoradiographic [^{14}C]deoxyglucose technique, as used previously in studies of central ET responses (6,12–19), a selective analysis restricted to a total of six related regions was performed in the present work. Other than the caudate nucleus located near the injection site, additional brain structures in the autoradiographic analysis were chosen specifically because of a) their previous demonstration of metabolic sensitivity to ICV ET (12,15); b) their neural connectivity via mono- or polysynaptic projections from the caudate nucleus or other likely origin sites of metabolic stimulation in the borders of the injected lateral ventricle, such as the lateral septal nucleus and hippocampal formation; and c) their relatively high binding affinities for [^{125}I]ET-1 (9,24). Three structures, the medial terminal nucleus of the accessory optic tract and the two cerebellar cortical subregions—paramedian lobular cortex and copula pyramis—are particularly enriched with ET receptors (24) and so may be extraordinarily sensitive to circuit stimulation by ICV ET. Although other structures included in our previous analyses fit the above criteria (12–15,18,19), we limited the present investigation to the six regions indicated to facilitate interpretations regarding the dose-response relationships and behavioral and physiological changes anticipated.

The following figure numbers refer to those in the neuroanatomical atlas of Swanson (39). The periventricular margin of the caudate nucleus was analyzed at the level of the tract made by the injection cannula, Figs. 20–22. The pars reticulata of the substantia nigra and medial terminal nucleus of the accessory optic tract were analyzed in the region of Figs. 36–38. Figures 61–65 provided reference for the locations of the inferior olive rostral lamella and cerebellar paramedian lobule and copula pyramis.

Metabolic Morphometry

A morphometric analysis was also conducted on the area of metabolic stimulation specifically within the periventricular ipsilateral caudate nucleus. Under the control of the analyst, pixel densities inside and outside the region of stimulation were differentiated by a computer-defined line, and the region of elevated metabolic activity was assessed by morphometric discrimination. Pixel densities were determined by derivation of the rates of glucose metabolism using the operational equation of the [^{14}C]deoxyglucose technique. This analysis was conducted to test the hypothesis that ET would have dose-graded effects on the area of tissue stimulation radiating into the caudate nucleus from the ventricular border.

Statistics

Statistical analyses of the metabolic and physiological data were conducted using a one-way analysis of variance at the 0.05 level of significance. If significant F-ratios were found, a modified t-statistic was employed to discern intergroup differences (3). Behavioral data were compared using the Mann-Whitney U-test at $p < 0.05$.

RESULTS

Behavior and Physiology

To establish consistency for the group-to-group analysis, we obtained peak readings between 15 and 40 min after the ICV injections, at which time the maximal response to central ET occurred. For the most part, the behavioral markers did not reveal an ET effect at the 1.5 pmol dose, although sniffing and masticatory responses may indicate early signs of central

TABLE 1

BEHAVIORAL OBSERVATIONS ON RATS GIVEN VARIOUS INTRAVENTRICULAR DOSES OF ENDOTHELIN

	Endothelin Doses					
	Saline (12)	1.5 pmol (5)	3 pmol (10)	6 pmol (14)	9 pmol (13)	18 pmol (5)
Barrel rolling (BR)	0/12	0/5	4/10	12/14	13/13	5/5
BR latency (min)	–	–	7.0 ± 0.7*†	5.0 ± 0.3*†‡	4.6 ± 0.3*†‡	3.6 ± 0.2*†‡§
Body twisting	0	0	2.0 ± 0.5	2.9 ± 0.1*†‡	3.0 ± 0.0*†‡	3.0 ± 0.0*†‡
Nystagmus	0	0	0.8 ± 0.6*†	2.8 ± 0.1†‡	2.9 ± 0.1*†‡	3.0 ± 0.0*†‡
Facial clonus	0	0	0.4 ± 0.4	1.9 ± 0.3*†‡	2.3 ± 0.3*†‡	2.8 ± 0.2*†‡§
Forelimb clonus	0	0	1.2 ± 0.5*†	2.3 ± 0.3*†	2.3 ± 0.4*†	2.6 ± 0.2*†‡
Forepaw dystonia	0	0	1.2 ± 0.7	2.5 ± 0.3*†	2.6 ± 0.2*†	2.8 ± 0.2*†
Tail extension	0	0.4 ± 0.2	1.4 ± 0.5*†	1.8 ± 0.8*†	3.0 ± 0.0*†‡§	3.0 ± 0.0*†‡§
Sniffing	1.4 ± 0.2	2.4 ± 0.2*	2.4 ± 0.6	2.1 ± 0.4	2.9 ± 0.1*†	3.0 ± 0.0*†§
Piloerection	1.2 ± 0.4	1.8 ± 0.4	2.4 ± 0.6	2.5 ± 0.3	2.4 ± 0.4	2.4 ± 0.4
Exophthalmos	0.8 ± 0.3	1.6 ± 0.5	2.4 ± 0.2*	2.8 ± 0.2*†	2.8 ± 0.1*†	2.8 ± 0.2*
Vocalization	0	0	0.4 ± 0.4	0.9 ± 0.3	0.9 ± 0.2	1.6 ± 0.2*†
Mastication	1.0 ± 0.4	2.4 ± 0.2*	2.2 ± 0.3	2.1 ± 0.3	2.3 ± 0.3*	0.8 ± 0.5

Behavioral assessments were made on a response scale of 0–3: 0 = absent, 1 = mild, 2 = moderate, 3 = severe. All values are means ± SE for number of rats in parentheses. Values were taken during peak response that occurred between 20–40 min after ICV administration of solution (except rats given 18 pmol ET, which was lethal within 10–20 min; see the Results section).

Statistical analyses: *differs from saline; †differs from 1.5 pmol ET; ‡differs from 3 pmol ET; §differs from 6 pmol ET; ¶differs from 9 pmol ET; #differs from 18 pmol. All volumes ≤ 3 μl.

TABLE 2

PHYSIOLOGICAL DATA FOR RATS GIVEN VARIOUS INTRAVENTRICULAR DOSES OF ENDOTHELIN

	Endothelin Doses					
	Saline (12)	1.5 pmol (5)	3 pmol (10)	6 pmol (14)	9 pmol (13)	18 pmol (5)
Physiological indices sensitive to endothelin						
Arterial blood pressure						
Systolic (mmHg)	162 ± 3	158 ± 4	161 ± 10	177 ± 6	184 ± 5*†‡	232 ± 10*†‡§
Diastolic (mmHg)	110 ± 8	100 ± 3	103 ± 9	117 ± 3†	121 ± 5†‡	134 ± 6*†‡§
Mean (mmHg)	127 ± 5	118 ± 3	122 ± 9	138 ± 4*†‡	141 ± 4*†‡	167 ± 7*†‡§¶
Arterial blood analysis						
PCO_2 (mmHg)	36 ± 1	32 ± 2	29 ± 1*	27 ± 2*†	26 ± 2*†	29 ± 2*
pH	7.45 ± 0.01	7.47 ± 0.02	7.50 ± 0.01*	7.52 ± 0.02*†	7.52 ± 0.01*†	7.50 ± 0.02*
Plasma glucose (mg/dl)	151 ± 6	171 ± 13	174 ± 7	189 ± 9	244 ± 22*†‡§#	169 ± 8
Physiological indices not sensitive to endothelin						
Heart rate (beats/min)	445 ± 22	469 ± 22	421 ± 65	489 ± 9	481 ± 11	431 ± 53
Hematocrit	45 ± 1	42 ± 1	46 ± 2	45 ± 1	46 ± 1	46 ± 1
Pulse pressure (mmHg)	59 ± 2	63 ± 3	62 ± 4	61 ± 2	68 ± 4	98 ± 8*†‡§¶

All values are means ± SE for number of rats in parentheses. Volumes ≤ 3 μl. Values were taken during peak response that occurred between 20–40 min after ICV administration of solution (except rats given 18 pmol ET, which was lethal within 10–20 min; see the Results section).

Statistical analyses ($p < 0.05$): *differs from saline; †differs from 1.5 pmol ET; ‡differs from 3 pmol ET; §differs from 6 pmol ET; ¶differs from 9 pmol ET; # differs from 18 pmol.

ET activity at this ET concentration (Table 1). At the 3 pmol ET dose, which appears to be at the threshold concentration for eliciting barrel rolling (4 out of 10 animals responding within 7 min of injection), the first conventional signs of convulsive activity appeared, including significant grades for nystagmus, exophthalmos, forelimb clonus, and tail rigidity (Table 1). A characteristic of ET-induced convulsions in these rats immobilized at the hindquarters was a static twisted position of the forebody under the long axis produced by ICV doses of ET > 3 pmol.

At 6 and 9 pmol ET injections, the latency to barrel rolling was reduced from 7.0 min at the 3 pmol dose to 5.0 min

TABLE 3

EFFECTS OF VARIOUS INTRAVENTRICULAR DOSES OF ENDOTHELIN ON RATES OF GLUCOSE METABOLISM

	Saline (7)	3 pmol (5)	Percent Increase From Saline	6 pmol (5)	Percent Increase From Saline	9 pmol (6)	Percent Increase From Saline	Critical Difference ($p < 0.05$)
Injection site								
Caudate nucleus	0.69 ± 0.05	1.17 ± 0.17*	70	1.14 ± 0.12*	65	1.49 ± 0.11*†‡	116	0.25
Midbrain and medulla oblongata								
Substantia nigra pars reticulata	0.49 ± 0.03	0.53 ± 0.07	NS	0.66 ± 0.15	NS	1.09 ± 0.10*†‡	122	0.23
Medial terminal nucleus[a]	0.47 ± 0.03	0.45 ± 0.03	NS	0.50 ± 0.09	NS	0.79 ± 0.10*†‡	68	0.20
Inferior olive, rostral lamella	0.73 ± 0.03	0.65 ± 0.04	NS	0.84 ± 0.15	NS	1.07 ± 0.15*†‡	47	0.22
Cerebellar cortex								
Paramedian lobule	0.52 ± 0.04	0.53 ± 0.06	NS	0.62 ± 0.10	NS	0.99 ± 0.04*†‡	90	0.17
Copula pyramis	0.58 ± 0.04	0.74 ± 0.10*	28	0.79 ± 0.09*	36	1.09 ± 0.09*†‡	88	0.17

Values are means ± SE for number of rats in parentheses. Units for rates of glucose metabolism are $\mu mol\ g^{-1}\ min^{-1}$ for structures ipsilateral to the intraventricular injection. All volumes ≤ 3 μl.

[a]Accessory optic tract. % Increase from Saline was calculated by (Endothelin ipsilateral response − saline ipsilateral response ÷ saline ipsilateral response) × 100. The statistical analysis provides significant or nonsignificant numerical changes based on multiple comparison tests deriving "critical differences" for significant F-ratios.

*Higher than corresponding value in saline group, $p < 0.05$.

$p < 0.10$. †‡Higher than corresponding values in 3 and 6 pmol ET groups, respectively, $p < 0.05$.

NS, not significant ($p > 0.05$).

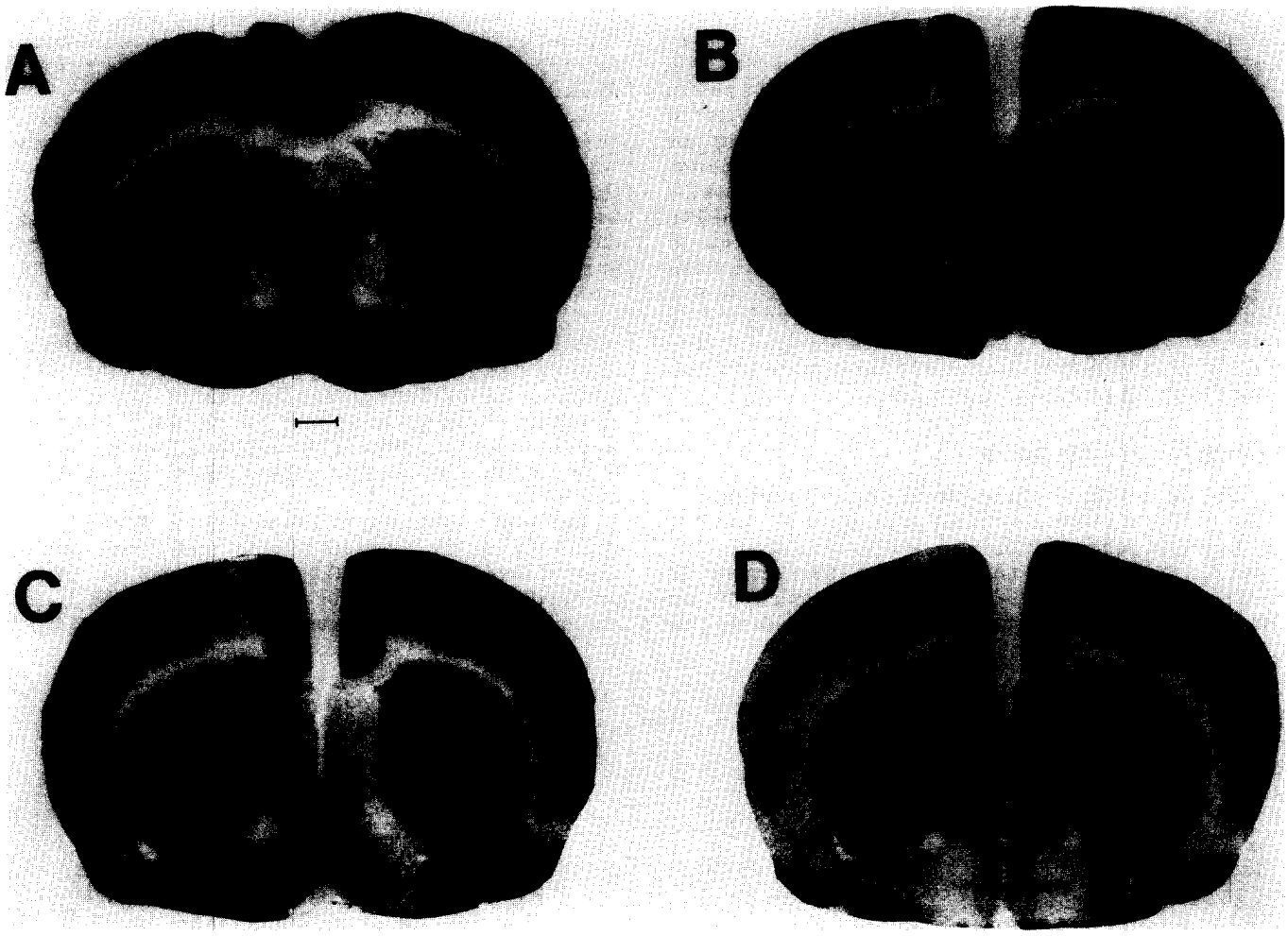

FIG. 1. [^{14}C]Deoxyglucose autoradiographs illustrating metabolic responses to lateral ventricular injection (thick vertical arrow in A indicates the cannula tract) of saline (A) or ET (3, 6, 9 pmol in B–D, respectively). cn, caudate nucleus; sp, lateral septal nucleus. Rates of glucose metabolism are proportional to the darkness in the image. Bar under A = 1 mm.

(Table 1). Compared to the 3 pmol injection, the barrel-rolling incidence was high or absolute in 6–9 pmol-injected rats, the animals became tonic in the upper trunk twisted position, and several other kinds of behavioral stimulation occurred (Table 1). The most reliable behavioral markers for an ET-induced central effect were barrel rolling itself, sniffing, nystagmus, exophthalmos, facial and forelimb clonic activity, dystonia of the forepaws, tail extension, and the static twisted position of the torso. Other behavioral signs, such as piloerection and vocalization, indicated a dose–response trend, but were not as reliable as those markers discussed above. The severe gradings for the 18 pmol ET injection reflect the strong response within 15 min of injection of the peptide, which was lethal at this dose.

Six of the nine physiological variables proved to be dose sensitive to ICV ET (see Table 2). At the 1.5 and 3 pmol ET doses, there was an increasing trend in systolic, diastolic, and mean arterial pressures compared to the saline control condition, although this trend was not statistically significant ($p > 0.05$). At 6 pmol ET, however, mean arterial pressure was significantly elevated ($p < 0.05$) compared to the saline group, 1.5 pmol ET, or 3 pmol ET (Table 2). Arterial PCO_2 and pH did not change significantly from the responses to saline until the 3 pmol ET dose, while both the 6 and 9 pmol ET doses produced greater hyperventilatory responses affecting the PCO_2 and pH measurements than those to saline or 1.5 pmol ET (Table 2). The threshold for significant changes in arterial PCO_2 and pH, therefore, appeared to occur between 1.5 and 3 pmol ET. The arterial plasma glucose concentration displayed an increasing trend toward a hyperglycemic condition that became statistically significant compared to saline only for the 9 pmol ET dose (Table 2). It should be noted that, at the 18 pmol ET dose, rats died between 10 and 20 min following injection from symptoms probably related to arterial hypertension and pulmonary edema, and so prevented us from describing the effects of this dose more completely. These rats displayed extraordinary pulse pressures 5–15 min after ICV injection (Table 2). The data showed in Table 2 for 18 pmol ET represent the largest effect observed before the animal's condition deteriorated.

Attempting to refine the physiological survey of responses to central ET, we partitioned the physiological parameters

into those proving to be effective indices of the ET response (Table 2) and those that were less or not effective (lower part of Table 2). To summarize, systolic, diastolic, and mean arterial pressures, arterial PCO_2 and pH, and the arterial plasma glucose concentration were sensitive discriminating indices of ET effects in the range of ICV ET doses tested. Heart rate (range between groups of 421–489 beats/min), pulse pressure, and arterial hematocrit (42–46%) did not vary significantly between the groups (bottom part of Table 2). Arterial PO_2 was normal (between 88 and 98 mmHg) for all groups of rats. As we reported previously (15), core temperature was significantly depressed by 9 pmol ICV ET over the course of an hour observation (35.4 ± 0.3°C at 20 min postinjection in this study). The other ET doses (1.5–6 pmol) produced body temperatures in the range of 35.3–35.8°C. Saline-injected rats had a mean temperature of 36.0 ± 0.2°C.

Cerebral Glucose Metabolism

Table 3 outlines the changes in the rates of glucose utilization for the six specific brain structures examined at four ICV test dosages: saline, and the 3, 6, and 9 pmol doses of ET. The 1.5 pmol dose was not used in this series because the physiological/behavioral series indicated its relatively low level or absence of stimulation. The 18 pmol dose was not employed because of its extreme pathophysiological stimulation and lethality within 20 min, conditions precluding quantitative application of the [^{14}C]deoxyglucose method (requiring 45 min).

In the analysis of variance for rates of glucose metabolism in individual structures across the treatment groups, each structure of the present report produced a significant *F*-ratio that was followed by application of multiple comparison statistics (derivation of critical differences) to discern intergroup differences. The periventricular caudate nucleus had a 70% increase ($p < 0.05$) and the copula pyramis a 28% increase ($p < 0.10$) in glucose metabolism with the lowest dose of ET tested, 3 pmol (Figs. 1, 3, and 4). No other structures demonstrated significant stimulation at the 3 pmol ET concentration (Table 3) (Figs. 1–3). At the 6 pmol dose of ET, which evoked convulsive activity (Table 1) and a high degree of physiological stimulation (Table 2), the caudate nucleus (Figs. 1 and 4) and cerebellar copula pyramis (Figs. 3 and 4) had significantly elevated metabolic activity (range of 36–65%, $p < 0.05$) (Fig. 4).

The trend for the other structures analyzed, although not statistically significant, was for higher rates of glucose metabolism at the 6 pmol dose (Table 3). At 9 pmol ET, which produced strong, consistent convulsive responses (Table 1), all structures analyzed had substantially increased rates of glu-

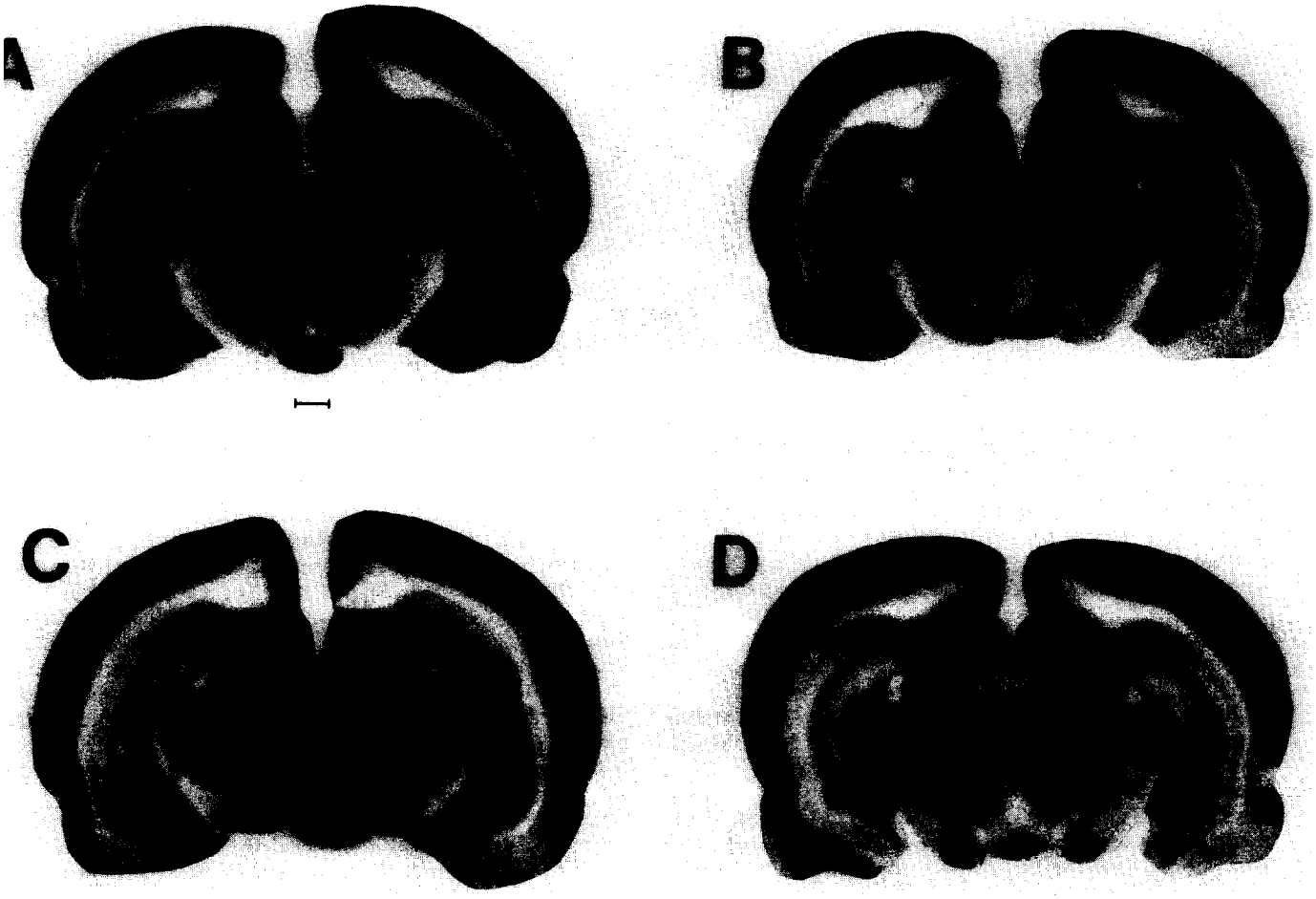

FIG. 2. [^{14}C]Deoxyglucose autoradiographs illustrating metabolic responses in the substantia nigra pars reticulata (snr) to lateral ventricular injection of (A) saline, (B) 3 pmol ET, (C) 6 pmol ET, and (D) 9 pmol ET. Rates of glucose metabolism are proportional to the darkness in the image. Bar under A = 1 mm.

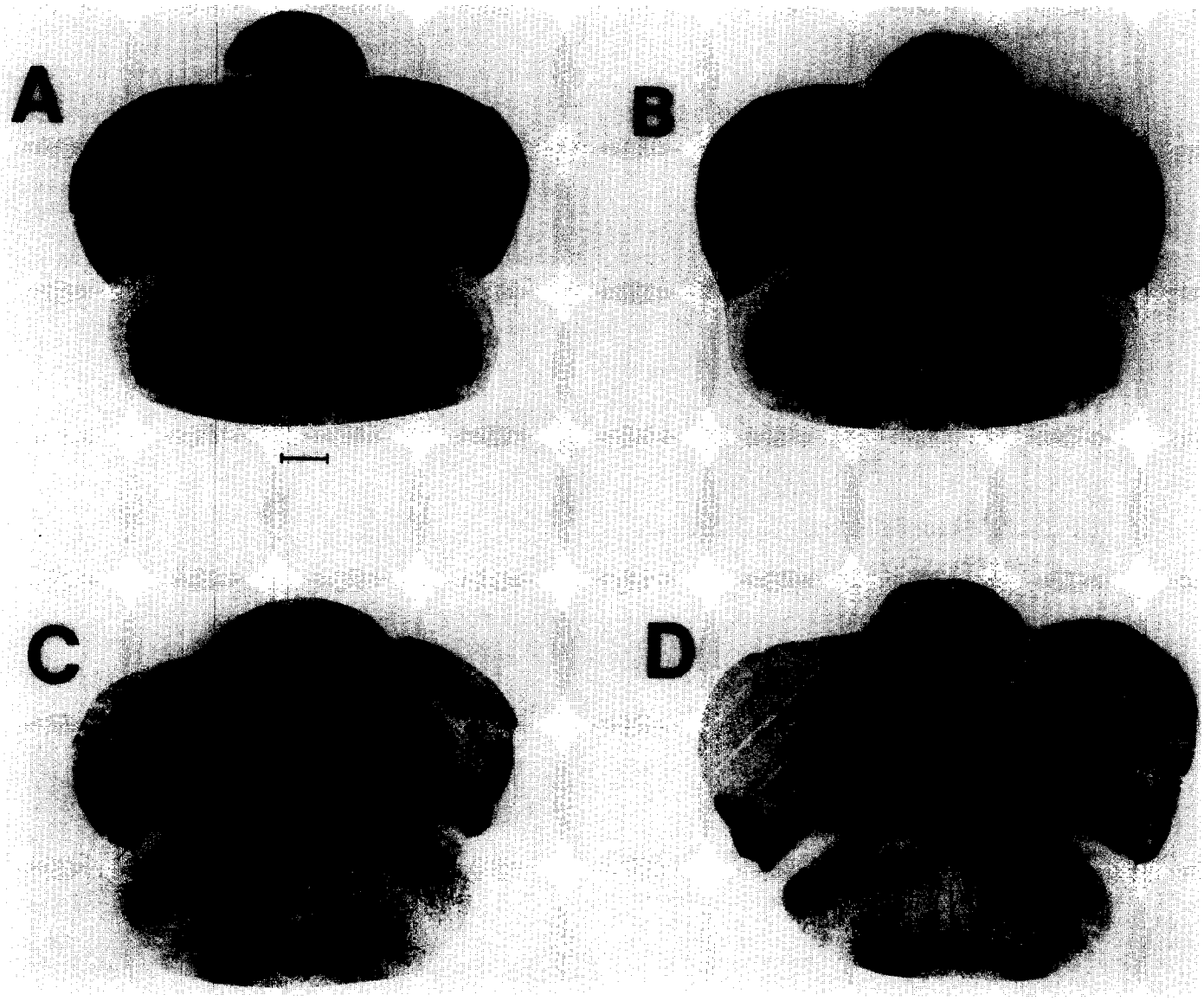

FIG. 3. [^{14}C]Deoxyglucose autoradiographs illustrating metabolic responses in the cerebellar copula pyramis (cp) and inferior olivary complex (io) following lateral ventricular injection of (A) saline, (B) 3 pmol ET, (C) 6 pmol ET, and (D) 9 pmol ET. Rates of glucose metabolism are proportional to the darkness in the image. Bar under A = 1 mm.

cose utilization (Table 3; Figs. 1–3). The largest increases occurred in the caudate nucleus and substantia nigra pars reticulata (about 120%) (Figs. 1, 2, and 4), with slightly weaker stimulation in the two subregions of cerebellar cortex (88–90%) (Fig. 3), and smaller increases in the optic medial terminal nucleus (68%) and inferior olive (47%) (Table 3) (Figs. 2 and 3). All these high metabolic rates produced by 9 pmol ET exceeded those of the lower doses, indicating a vigorous dose-response effect.

Metabolic Morphometry

The periventricular caudate nucleus exhibited stimulated rates of glucose metabolism in a homogeneous pattern which, by computerized morphometric analysis, proved to be an area of consistent dimension with each ET dose (e.g., Fig. 5). Whereas saline had no periventricular effect, the three ET doses (3, 6, and 9 pmol) displayed similar pixel areas of increased optical density (i.e., elevated glucose metabolism) ranging from 7.2 to 7.7 $\times$ 10^5 μm^2 (range of SE = 1.7–1.9 $\times$ 10^5 μm^2) ($p > 0.05$). Thus, the numbers of pixels (morphometrically determined areas) with increased optical density were the same between groups, but the optical densities (rates of glucose metabolism) were dose related.

DISCUSSION

The main finding of this investigation was the identification of a threshold and range of ET doses producing behavioral, physiological, and cerebral metabolic effects. At the 3 pmol dose of ET, the arterial PCO_2 was reduced and the pH increased significantly by hyperventilation, barrel rolling, torso twisting, and forelimb clonic activity were evident, and a hypermetabolic response was manifested in the periventricular caudate nucleus. More intense stimulatory effects and a wider range of hypermetabolic activity in the brain occurred at the 6

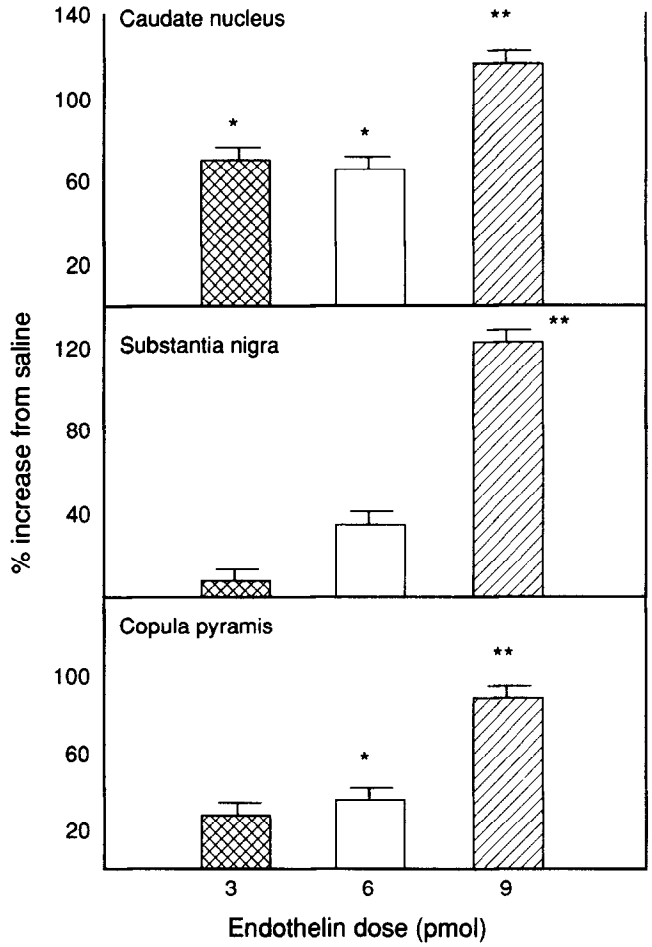

FIG. 4. Histograms summarizing the increases in metabolic activity at three levels of the neuraxis following lateral ventricular injection of ET in the doses shown on the abscissa. Values expressed are the mean percent increases from the control (saline) injection (± SE) in the respective structures. The values were calculated by the equation: % increase in rate of glucose utilization = [(endothelin ipsilateral response − saline ipsilateral response) ÷ saline ipsilateral response] × 100. *Significant difference from saline condition ($p < 0.05$). **Significant difference from 3 and 6 pmol ET conditions ($p < 0.05$).

and 9 pmol levels of ICV ET. The 18 pmol dose caused the most severe physiological and behavioral responses acutely, but was lethal due to arterial hypertension and pulmonary edema within 20 min of ICV injection. The results, therefore, confirm the preliminary findings of Moser and Pelton (32) and Ouchi and colleagues (36), establishing these ICV doses of ET as a graded model of peptide-induced brain stimulation, physiological activation, and motor convulsions.

We shall interpret the behavioral and physiological findings together and with respect to the diversity of metabolic stimulation in the brain. We shall also speculate about the neural circuit activation that results from ICV injection of ET doses between 3 and 9 pmol.

Behavioral and Physiological Effects of Central ET

We previously proposed that the barrel-rolling disturbance caused by ICV ET involves specifically the visuovestibular and oculomotor systems (12) which a) contain relatively high densities of ET receptors (9,24), b) are neurally connected with periventricular nuclei of the injected lateral ventricle, and c) have selectively stimulated rates of glucose metabolism revealed by [^{14}C]deoxyglucose autoradiography (12). Confirming previous behavioral analyses (32), we found in the present work that the threshold for the cerebral response to ET is at 3 pmol. Compared to the limited cerebral effects of 1.5 pmol ET, this dose is associated with barrel rolling, the tonically twisted position of the upper trunk, limb clonus, nystagmus, exophthalmos, sniffing, and tail rigidity. All of these signs were more severe as the ET dose increased above 3 pmol. The general behavioral response was consistent with the physiological results, revealing a sympathetically activated animal.

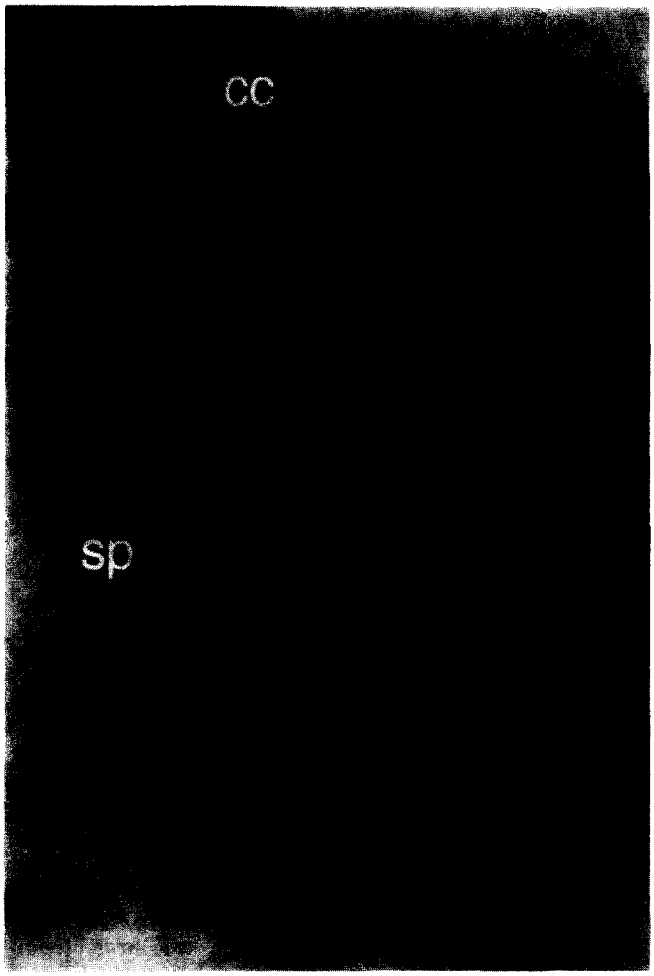

FIG. 5. Magnified (×50) image of the periventricular region metabolically stimulated by lateral ventricular injection of 9 pmol ET. The dotted line describes the morphometrically analyzed area of increased optical density (increased rates of glucose metabolism) among pixels in the stimulated subregion of the caudate nucleus (cn). Other structures are the corpus callosum (cc) and lateral septal nucleus (sp). The lateral ventricle is the space beneath cc. The position of the image was confirmed by overlay with a digitized matching histological section. The area of caudate stimulation, approximately 7.5 × 10^5 μm^2, did not differ according to dose of ET injected (see the Results section and the Metabolic Morphometry section).

The present dose-response behavioral findings with ET are interesting in comparison to those obtained with other peptides and amines. The substances listed in Table 4 have a wider dose effective range and much higher threshold doses causing behavioral stimulation than does ET. By comparison of the lowest doses to evoke barrel rotation by ICV injection, ET (3 pmol) is more potent than vasopressin by 300 times (22), neuropeptide Y by 1000 times (23), somatostatin by 3000 times (1,5,8), and cholecystokinin by 8600 times (31). Compared to neuroactive amines or their analogs, ET is ≥ 100 times more potent than the glutamatergic agonists, domoic acid, *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), or kainic acid (7). Using barrel rotation as the index of central effect, these comparisons prove ET as the most powerful neurostimulant yet described in vivo.

Among nine physiological variables assessed, the arterial PCO_2 and pH proved to be the only measurements significantly changed at the 3 pmol dose of ET, the lowest dose producing statistically significant physiological effects compared to the 1.5 pmol dose or saline condition. Previous investigators (28,33,34,46) identified that central ET evokes systemic sympathetic activation and vasopressin release, probably by stimulation of third ventricular hypothalamic structures affecting pituitary function and regulating sympathetic outflow to the periphery. In a foregoing study, we showed that several nuclei of the hypothalamus were metabolically stimulated by 9 pmol ET injected into a lateral ventricle (13). We speculated that these structures were the neural substrates initiating the pituitary and sympathetic responses to ICV ET injection (13,14). A key finding was that the distribution of Evans blue dye combined in the lateral ventricular ET injection penetrated to tissues bordering the third cerebral ventricle. Therefore, it is likely that ET injected into a lateral ventricle contacts hypothalamic nuclei bordering the third ventricle and initiates an increase in the functional activity of these cell groups that project to the pituitary gland and medulla oblongata to influence hormone secretion and sympathetic outflow. It is also possible that ET elicits a hyperventilatory response by activation of neural efferent projections from third ventricular hypothalamic structures to other forebrain and brain stem regions involved in systemic physiological regulation that were not evaluated in our analyses.

TABLE 4

COMPARISON OF THRESHOLD INTRAVENTRICULAR DOSES OF NEUROACTIVE PEPTIDES AND AMINO ACIDS VS. ENDOTHELIN FOR PRODUCING BARREL ROTATION

Neuroactive Agents	Molecular Weight	Threshold Dose for Barrel Rotation	Approximate Comparison to Endothelin
Endothelin	2492	3 pmol	
Peptides			
Vasopressin (25)	1084	920 pmol	300 : 1
Neuropeptide Y (23)	4272	5 nmol	1000 : 1
Somatostatin (8)	1638	9 nmol	3000 : 1
Cholecystokinin (31)	1143	26 nmol	9000 : 1
Amino acids and analogs			
Domoic Acid (7)	311	300 pmol	100 : 1
N-Methyl-D-Aspartate (7)	147	10 nmol	3300 : 1
AMPA (7)	186	10 nmol	3300 : 1
Kainic Acid (7)	213	10 nmol	3300 : 1
L-Aspartate* (41)	133	530 nmol	177000 : 1
L-Glutamate* (41)	146	580 nmol	193000 : 1

References in parentheses.
*Intracaudate injection.
AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid.

Cerebral Metabolic Stimulation and Neural Circuit Activation by Central ET

The metabolic investigation revealed the neuroactive effects of ventricular ET at three levels of the neuraxis—a) near the injection site in the lateral periventricular forebrain (caudate nucleus), b) at the midbrain level likely within a few synapses of the source of neural stimulation (substantia nigra pars reticulata, medial terminal nucleus of the accessory optic tract), and c) in the caudal brain stem and cerebellum, which are at polysynaptic distances from the origin of stimulation (rostral lamella of inferior olive, paramedian lobule, copula pyramis). We have discussed previously that the structures of this analysis contain appreciable numbers of ET receptors and are anatomically connected (6,13,15), facts that indicate a possible intact neural circuit under stimulation from the putative origin of ET effect in the periventricular caudate nucleus. Inspection of the distribution of dye in the lateral ventricular ET injectate proved that it likely penetrated the ipsilateral ventricle and the third ventricle, and was carried in the cerebral aqueduct to the level of the pons (15). Having specifically measured the rates of blood flow in periventricular structures and found no evidence for severe ischemic effects of ET (19), we believe that the responses recorded are primarily neural rather than vascular-derived effects. We speculate, therefore, that ET produces neural stimulation of periventricular structures that then transmit the increased activity efferently to their respective projection zones.

We had further hypothesized that the tissue area of metabolic stimulation within a given periventricular nucleus, such as the caudate nucleus, would be enlarged by increasing doses of ICV ET. This hypothesis proved to be untrue, however, since the region of stimulated tissue was equal for the three doses analyzed. However, the metabolic activity of neurons in this region was sensitive to ET dose. Thus, an ICV injection of ET at any dose appears to affect a consistent periventricular sphere of the caudate nucleus, probably by a similar degree of tissue diffusion. It appears unlikely that interneuronal circuits within the medial part of the caudate nucleus are metabolically activated by ICV ET to a degree detectable by deoxyglucose autoradiography.

A new finding in the present study was the demonstration of a dose-response relationship for the rates of glucose metabolism in the six structures analyzed. The metabolic responses at each of the submaximal doses, 3 and 6 pmol, were not uniform from structure to structure. The caudate nucleus, for example, showed significant metabolic stimulation at the 3 pmol dose, which produced barrel rolling and other convulsions. These observations may indicate importance for the caudate nucleus in the neural regulation of the proprioceptive and oculomotor disturbances related to barrel rolling [previous discussion, see (12)]. Balaban and colleagues suggested that neuropeptides (e.g., somatostatin, vasopressin) could stimulate brain circuits asymmetrically, causing the rat to perceive an imbalanced equilibrium leading to barrel rolling (2). The animal's effort to correct this imbalance results in symp-

toms characteristic of acute vestibulocerebellar dysfunction (ataxia, nystagmus, body sway), eventually progressing to barrel rolling (2).

Although our foregoing work had identified potent metabolic responses to ET in all the structures of the present analysis, the dose-response relationships revealed that the thresholds for metabolic stimulation in the medial terminal nucleus of the accessory optic tract, inferior olivary nucleus (rostral lamella), and paramedian cortex of the cerebellum were above 6 pmol ET. However, another ET-rich subregion of the cerebellar cortex, the copula pyramis, was sensitive to 3 pmol ET, as was the caudate nucleus, which is an ET receptor-poor structure (9,21,24). At the 6 to 9 pmol doses of ET, the caudate nucleus and cerebellar copula pyramis were strongly stimulated, correlating with more vigorous barrel-rolling and other convulsive signs. The substantia nigra pars reticulata, moderate in ET binding (24) and having increased metabolic activity across the range of ET doses, is implicated by previous work as a relay in several forms of seizure activity (11,29). It appears from these various findings that ET receptor density is not a prerequisite for sensitivity of metabolic responsiveness to centrally injected ET. Neural afferent inputs due to circuit activation likely have an important role in establishing local rates of tissue glucose metabolism. Previous studies identified that the ET-induced stimulatory responses in the brain depend on dihydropyridine-sensitive calcium channels (15,33), inhibition of ATP-dependent K^+ channels opened by nicorandil (33), the A subtype of ET receptor (17), and glutamatergic NMDA receptors (6). In a hypothetical mechanism that might not be directly related to binding with its own receptors, ET may insert its lipophilic tail into the lipid bilayer of neuronal membranes (42), possibly affecting cation fluxes that could heighten excitability, release excitatory transmitters, and increase neuronal energy metabolism.

In conclusion, the present work establishes a basis for interpreting the cerebral metabolic correlates of behavioral dysfunction and systemic physiological stimulation produced by the centrally injected peptide, ET, at low picomolar doses. The results are consistent with speculation that the caudate nucleus, substantia nigra pars reticulata, inferior olivary nuclei, and cerebellar cortex are integrated functionally as a stimulated circuit in the barrel-rolling disorder produced by lateral ventricular injection of ET. By comparison with the threshold doses of other neuroactive peptides and amino acids evoking behavioral and physiological responses upon lateral ventricular injection, the findings of the present study reveal ET as the most powerful stimulant of the brain yet described.

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REFERENCES

1. Balaban, C. D.; Fredericks, D. A.; Wurpel, J. N. D.; Severs, W. B. Motor disturbances and neurotoxicity induced by centrally administered somatostatin and vasopressin in conscious rats: Interactive effects of two neuropeptides. Brain Res. 445:117–129; 1988.
2. Balaban, C. D.; Starčević, V. P.; Severs, W. B. Neuropeptide modulation of central vestibular circuits. Pharmacol. Rev. 41:53–90; 1989.
3. Bruning, J. L.; Kintz, B. L. Computational handbook of statistics, 2nd ed. Glenview, IL: Scott, Foresman and Company; 1977.
4. Burke, R. E.; Fahn, S. Electroencephalographic studies of chlorpromazine methiodide and somatostatin-induced barrel rotation in rats. Exp. Neurol. 79:704–713; 1983.
5. Burke, R. E.; Fahn, S. Studies of somatostatin-induced barrel rotation in rats. Reg. Pept. 7:207–220; 1983.
6. Chew, B. H.; Weaver, D. F.; Balaban, C. D.; Gross, P. M. NMDA-mediated metabolic activation of the cerebellar cortex in behaving rats by the neuropeptide endothelin-1. Brain Res. 647: 345–352; 1994.
7. Chiamulera, C.; Costa, S.; Valerio, E.; Reggiani, A. Domoic acid toxicity in rats and mice after intracerebroventricular administration: Comparison with excitatory amino acid agonists. Pharmacol. Toxicol. 70:115–120; 1992.
8. Cohn, M. L.; Cohn, M. Barrel rotation induced by somatostatin in the nonlesioned rat. Brain Res. 96:138–141; 1975.
9. Davenport, A. P.; Morton, A. J. Binding sites for ^{125}I ET-1, ET-2, ET-3 and vasoactive intestinal contractor are present in adult rat brain and neurone-enriched primary cultures of embryonic brain cells. Brain Res. 554:278–285; 1991.
10. Ehrenreich, H.; Kehrl, J. H.; Anderson, R. W.; Rieckmann, P.; Vitkovic, L.; Coligan, J. E.; Fauci, A. S. A vasoactive peptide, endothelin-3, is produced by and specifically binds to primary astrocytes. Brain Res. 538:54–58; 1991.
11. Gale, K. Mechanisms of seizure control mediated by gamma-aminobutyric acid: Role of the substantia nigra. Fed. Proc. 44: 2414–2424; 1985.
12. Gross, P. M.; Beninger, R. J.; Shaver, S. W.; Wainman, D. S.; Espinosa, F. J.; Weaver, D. F. Metabolic and neuroanatomical correlates of barrel-rolling and oculoclonic convulsions induced by intraventricular endothelin-1: A novel peptidergic signaling mechanism in visuovestibular and oculomotor regulation? Exp. Brain. Res. 95:397–408; 1993.
13. Gross, P. M.; Wainman, D. S.; Chew, B. H.; Espinosa, F. J.; Weaver, D. F. Calcium-mediated metabolic stimulation of neuroendocrine structures by intraventricular endothelin-1 in conscious rats. Brain Res. 606:135–142; 1993.
14. Gross, P. M.; Wainman, D. S.; Espinosa, F. J. Differentiated metabolic stimulation of rat pituitary lobes by peripheral and central endothelin-1. Endocrinology 129:1110–1112; 1991.
15. Gross, P. M.; Wainman, D. S.; Espinosa, F. J.; Nag, S.; Weaver, D. F. Cerebral hypermetabolism produced by intraventricular endothelin-1 in rats: Inhibition by nimodipine. Neuropeptides 21: 211–223; 1992.
16. Gross, P. M.; Weaver, D. F. A new experimental model of epilepsy based on the intraventricular injection of endothelin. J. Cardiovas. Pharmacol. 22 (Suppl. 8):S282–S287; 1993.
17. Gross, P. M.; Weaver, D. F.; Ho, L. T.; Pang, J. J.; Espinosa, F. J.; Edvinsson, L. FR139317, a specific ETA-receptor antagonist, inhibits cerebral activation by intraventricular endothelin-1 in conscious rats. Neuropharmacology 33:1155–1166; 1994.
18. Gross, P. M.; Weaver, D. F.; Wainman, D. S.; Chew, B. H.; Espinosa, F. J.; Nag, S. Potent metabolic stimulation of septal gray and cerebral white matter in vivo by intraventricular endothelin and nitric oxide. Biochem. Biophys. Res. Commun. 190: 975–981; 1993.
19. Gross, P. M.; Zochodne, D. W.; Wainman, D. S.; Ho, L. T.; Espinosa, F. J.; Weaver, D. F. Intraventricular endothelin-1 uncouples the blood flow: Metabolism relationship in periventricular structures of the rat brain: Involvement of L-type calcium channels. Neuropeptides 22:155–165; 1992.
20. Hoyer, D.; Waeber, C.; Palacios, J. M. [^{125}I]Endothelin-1 binding sites: Autoradiographic studies in the brain and periphery of various species including humans. J. Cardiovas. Pharmacol. 13(Suppl. 5):S162–S165; 1989.

21. Jones, C. R.; Hiley, C. R.; Pelton, J. T.; Mohr, M. Autoradiographic visualization of the binding sites for [^{125}I]endothelin in rat and human brain. Neurosci. Lett. 97:276–279; 1989.
22. Kasting, N. W.; Veale, W. L.; Cooper, K. E. Convulsive and hypothermic effects of vasopressin in the brain of the rat. Can. J. Physiol. Pharmacol. 58:316–319; 1980.
23. Kerkerian-Le Goff, L.; Forni, C.; Samuel, D.; Block, A.; Dusticier, N.; Nieoullon, A. Intracerebroventricular administration of neuropeptide Y affects parameters of dopamine, glutamate, and GABA activities in the rat striatum. Brain Res. Bull. 28:187–193; 1992.
24. Kohzuki, M.; Chai, S. Y.; Paxinos, G.; Karavas, A.; Casley, D. J.; Johnston, C. I.; Mendelsohn, F. A. O. Localization and characterization of endothelin receptor binding sites in the rat brain visualized by in vitro autoradiography. Neuroscience 42: 245–260; 1991.
25. Kruse, H.; Van Wimersma Greidanus, T. J. B.; De Wied, D. Barrel rotation induced by vasopressin and related peptides in rats. Pharmacol. Biochem. Behav. 7:311–313; 1977.
26. Lecci, A.; Maggi, C.-A.; Rovero, P.; Giachetti, A.; Meli, A. Effect of endothelin-1, endothelin-3 and C-terminal hexapeptide, endothelin (16–21) on motor activity in rats. Neuropeptides 16: 21–24; 1990.
27. Lovenberg, W.; Miller, R. C. Endothelin: A review of its effects and possible mechanisms of action. Neurochem. Res. 15:407–417; 1990.
28. Matsumura, K.; Abe, I.; Ysuchihashi, T.; Tominaga, M.; Kobayashi, K.; Fujishima, M. Central effect of endothelin on neurohormonal responses in conscious rabbits. Hypertension 17:1192–1196; 1991.
29. McNamara, J. O. Kindling model of epilepsy. Adv. Neurol. 44: 303–318; 1986.
30. Miller, R. C.; Pelton, J. T.; Huggins, J. P. Endothelins: From receptors to medicine. Trends Pharmacol. Sci. 14:54–60; 1993.
31. Morency, M. A.; Ross, G. M.; Hesketh, D.; Mishra, R. K. Effects of unilateral intracerebroventricular microinjections of cholecystokinin (CCK) on circling behavior in rats. Peptides 8:989–995; 1987.
32. Moser, P. C.; Pelton, J. T. Behavioral effects of centrally administered endothelin in the rat. Br. J. Pharmacol. 96:347; 1989.
33. Nishimura, M.; Takahashi, H.; Matsusawa, M.; Ikegaki, I.; Nakanishi, T.; Hirabayashi, M.; Yoshimura, M. Intracerebroventricular injections of endothelin increase arterial pressure in conscious rats. Jpn. Circ. J. 54:662–670; 1990.
34. Nishimura, M.; Takahashi, H.; Matsusawa, M.; Ikegaki, I.; Sakamoto, M.; Nakanishi, T.; Hirabayashi, M.; Yoshimura, M. Chronic intracerebroventricular infusions of endothelin elevate arterial pressure in rats. J. Hypertens. 9:71–76; 1991.
35. Niwa, M.; Kawaguchi, T.; Fujimoto, M.; Kataoka, Y.; Taniyama, K. Receptors for endothelin in the central nervous system. J. Cardiovas. Pharmacol. 17(Suppl. 7):S137–S139; 1991.
36. Ouchi, Y.; Kim, S.; Souza, A. C.; Iijima, S.; Hattori, A.; Kurihara, H.; Yazaki, Y. Central effect of endothelin on blood pressure in conscious rats. Am. J. Physiol. 256:H1747–H1751; 1989.
37. Simonson, M. S.; Dunn, M. J. The molecular mechanisms of cardiovascular and renal regulation by endothelin peptides. J. Lab. Clin. Med. 119:622–639; 1992.
38. Sokoloff, L.; Reivich, M.; Kennedy, C.; Des Rosier, M. H.; Patlak, C. S.; Pettigrew, K. D.; Sakurada, O.; Shinohara, M. The [^{14}C]deoxglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. J. Neurochem. 28:897–916; 1977.
39. Swanson, L. W. Brain maps: Structure of the rat brain. Amsterdam: Elsevier; 1992.
40. Takahashi, K.; Ghatei, M. A.; Jones, P. M.; Murphy, J. K.; Lam, H.-C.; O'Halloran, D. J.; Bloom, S. R. Endothelin in human brain and pituitary gland: Presence of immunoreactive endothelin, endothelin messenger ribonucleic acid and endothelin receptors. J. Clin. Endocrinol. Metab. 72:693–699; 1991.
41. Toth, E.; Lajtha, A. Motor effects of intracaudate injection of excitatory amino acids. Pharmacol. Biochem. Behav. 33:175–179; 1989.
42. Weaver, D. F.; Kim, S.; Gross, P. M. Theoretical conformational analyses of endothelin-1 in vacuum, aqueous, and lipid environments. J. Cardiovas. Pharmacol. 22:S374–S376; 1993.
43. Wurpel, J. N. D.; Dundore, R. L.; Barbella, Y. R.; Balaban, C. D.; Keil, L. C.; Severs, W. B. Barrel rotation evoked by intracerebroventricular vasopressin injections in conscious rats. II. Visual/vestibular interactions and efficacy of antiseizure drugs. Brain Res. 365:30–41; 1986.
44. Wurpel, J. N. D.; Dundore, R. L.; Barbella, Y. R.; Balaban, C. D.; Keil, L. C.; Severs, W. B. Barrel rotation evoked by intracerebroventricular vasopressin injections in conscious rats. I. Description and general pharmacology. Brain Res. 365:21–29; 1986.
45. Wurpel, J. N. D.; Dundore, R. L.; Bryan, R. M.; Keil, L. C.; Severs, W. B. Regional cerebral glucose utilization during vasopressin-induced barrel rotations or bicuculline-induced seizures in rats. Pharmacology 36:1–8; 1988.
46. Yamamoto, T.; Kimura, T.; Ota, K.; Shoji, M.; Inoue, M.; Sato, K.; Ohta, M.; Yoshinaga, K. Central effects of endothelin-1 on vasopressin release, blood pressure, and renal solute excretion. Am. J. Physiol. 262:E856–E862; 1992.
47. Yanagisawa, M.; Kurihara, H.; Kimura, S.; Tomobe, Y.; Kobayashi, M.; Mitsui, Y.; Yazaki, Y.; Goto, K.; Masaki, T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332:411–415; 1988.
48. Yanagisawa, M.; Masaki, T. Molecular biology and biochemistry of the endothelins. Trends Pharmacol. Sci. 10:374–378; 1989.