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Short communication

Functional correlates of repeated administration of cocaine and apomorphine in the rat

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Abstract

The [^{14}C]2-deoxyglucose method was applied to measure the effects of repeated (8 consecutive days) administration of apomorphine (0.5 mg/kg/day s.c.) or cocaine (15 mg/kg/day i.p.) on cerebral glucose utilization in freely moving rats. Altered rates of glucose utilization were measured in extrapyramidal motor areas, such as the globus pallidus, entopeduncular nucleus, subthalamic nucleus, substantia nigra and lateral habenula of both cocaine- and apomorphine-treated rats. Furthermore, cocaine-treated animals displayed increased glucose metabolism in the mesolimbic dopaminergic projections, such as nucleus accumbens and olfactory tubercle, and in the hippocampus. These results suggest that altered functional activity within the dopaminergic mesolimbic system may play a role in the process of sensitization to psychomotor stimulant drugs.

Keywords: Cocaine; Apomorphine; Deoxyglucose; Brain metabolism; Nucleus accumbens

1. Introduction

Dopamine receptor agonist drugs can be divided into two classes, psychomotor stimulant drugs and non-psychomotor stimulant drugs, based on behavioral and pharmacological criteria. Regardless of their psychomotor stimulant properties, repeated administration of dopamine receptor agonist drugs produces a progressive enhancement on motor activity and stereotypies (Kalivas et al., 1988; Rowlett et al., 1991). For instance, cocaine, which is readily self-administered by experimental animals, has motor effects similar to those of apomorphine, which lacks any incentive motivational property. The question arises as to whether different functional changes occur during the process of sensitization to psychostimulant and non-psychostimulant dopamine receptor agonists. Because of the close relationship between energy metabolism and functional activity in the central nervous system, the [^{14}C]2-deoxyglucose method (Sokoloff et al., 1977) for the measurement of local rates of cerebral glucose utilization is a potent tool with which to investigate the alterations in brain function produced by pharmacological treatments. The method has been widely applied to map the effects of the acute administration of dopamine receptor agonist drugs in experimental animals (Orzi et al., 1993). In the present study the [^{14}C]2-deoxyglucose procedure was used to measure the alterations of cerebral energy metabolism associated with the repeated administration of cocaine or apomorphine in freely moving rats.

2. Materials and methods

2.1. Animals

Experiments were performed on male Sprague-Dawley rats (Harlan-Nossan, Italy) weighing 270–320 g. Animals were housed in single cages and maintained

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under standard conditions of temperature (22 ± 1 °C) on a 12 h light/dark cycle (light on 07.00–19.00 h). They had free access to food and water.

2.2. Drug administration

Cocaine-HCl and apomorphine-HCl (Sigma, USA) were dissolved in saline just prior to use, at a concentration of 15 mg/ml and 0.5 mg/ml, respectively. Animals were divided into three groups, and administered with either cocaine (15 mg/kg intraperitoneal, $n = 5$), apomorphine (0.5 mg/kg subcutaneous, $n = 6$), or vehicle (1 ml/kg intraperitoneal, $n = 5$). Drugs or vehicle were given once daily for 8 consecutive days. All injection were performed between 9 a.m. and 12 a.m. The [^{14}C]2-deoxyglucose procedure was carried out on the day of the last injection.

2.3. Behavioral testing

To evaluate the effects of the different treatments on locomotor activity, animals were placed in an open-field Perspex activity cage (Basile, Italy) soon after each injection. Locomotor activity was measured by the number of 'bridges' that each animal made or broke with its paw between the 30 evenly spaced stainless steel bars of the cage floor. Counts were recorded every 10 min during the 50 min following each administration of drugs or vehicle. In addition, the occurrence of stereotypies and other behavioral changes was recorded by an observer not aware of the specific treatment.

2.4. Local cerebral glucose utilization

On the day of the experiment, each animal was lightly anesthetized with halothane (2% in oxygen), and polyethylene catheters were inserted into one femoral artery and vein, then run subcutaneously to exit at the nape of the neck. Such placement of the catheters allows animals to behave freely during the experimental procedure (Crane and Porrino, 1989). Animals were allowed at least 3 h for recovery from anesthesia and surgery, before receiving the last injection of drugs or vehicle according to the above schedule. The [^{14}C]2-deoxyglucose experimental procedure was begun 10 min after the last administration of cocaine and vehicle, and 5 min after the last administration of apomorphine, respectively. Time intervals between administration of the drugs and measurement of glucose utilization were established on the basis of previous reports (McCulloch et al., 1982; London et al., 1986; Porrino, 1993), in order to maximize the effectiveness of the [^{14}C]2-deoxyglucose method in identifying changes of functional activity. The procedure was carried out according to the original description (Sokoloff et al., 1977). Briefly, a pulse of [^{14}C]2-deoxyglucose (100 μCi/kg, specific activity 50–55 mCi/mmol, Amersham, UK) was injected through the venous catheter. Timed arterial blood samples were then collected and centrifuged immediately. Plasma ^{14}C concentrations were determined by liquid scintillation counting (LKB Rackbeta, Italy), and plasma glucose concentrations were assayed by means of the Beckman II Glucose Analyzer (Beckman, USA). Approximately 45 min after the administration of the tracer, animals were killed by the i.v. administration of a lethal dose of sodium pentobarbital. Brains were rapidly removed and frozen in isopentane at −40°C, then stored in a freezer at −70°C, until sectioning. Coronal brain sections (20 μm) were cut in a cryostat maintained at −21°C. Sections were picked up on glass coverslips and dried on a hot plate (+60°C), then autoradiographed on Kodak Min-R X-ray films (Kodak, Italy), along with a set of calibrated [^{14}C]plastic standards (Amersham, UK). Films were exposed for 14 days, and developed automatically. Autoradiograms were analyzed by quantitative densitometry using a computerized image-processing system (MCID, Imaging Research, Canada). Optical density measurements for each brain area, identified according to rat brain atlas of Pellegrino et al. (1979), were made in at least five sections. Within the nucleus accumbens, the 'shell' and 'core' (dorsomedial) subportions were identified as previously described (Pontieri et al., 1994). Tissue ^{14}C concentrations were determined from the optical densities and a calibration curve obtained from densitometric analysis of the autoradiograms of the calibrated standards. Rates of glucose utilization were then calculated from the local ^{14}C tissue concentrations, the time-course of the plasma glucose and ^{14}C concentrations, and the appropriate constants according to the operational equation of the method (Sokoloff et al., 1977).

2.5. Physiological parameters

Approximately 20 min after the administration of drugs or vehicle, mean arterial blood pressure was determined by means of an air-damped mercury manometer. Arterial plasma glucose concentrations were assayed during the [^{14}C]2-deoxyglucose experimental procedure, as described above.

2.6. Statistical analysis

Local rates of cerebral glucose utilization were measured in 42 discrete brain areas. Statistical analysis was carried out on each portion individually by means of a one-way analysis of variance followed by Bonferroni's t-test for multiple comparisons. Locomotor activity and physiological parameters were similarly analyzed.

3. Results

3.1. Behavior

Repeated administration of cocaine and apomorphine produced a progressive increase of motor activity. Cocaine-treated animals displayed rearing and head bobbing, whereas apomorphine-treated rats showed head bobbing and short-range stereotypic activity of the forelimbs. In either case, the time interval between the administration of the drug and the appearance and intensity of behavioral alterations shortened with the progression of treatment.

Table 1
Effects of the repeated administration of apomorphine and cocaine on local rates of cerebral glucose utilization (μmol/100 g/min) in freely moving rats

Structure	Vehicle ($n=5$)	Apomorphine ($n=6$)	Cocaine ($n=5$)
Nucleus accumbens shell	88±2	86±3	104±6 [a,b]
Nucleus accumbens core	74±6	88±2	95±6 [a]
Olfactory tubercle	77±2	85±4	95±6 [a]
Medial prefrontal cortex	66±3	74±2	74±4
Anterior cingulate cortex	93±5	99±4	111±6
Lateral septum	53±1	61±3	63±5
Medial septum	69±3	90±3 [a]	90±3 [a]
Hippocampus CA1	64±2	63±5	76±3
Hippocampus CA2	68±2	69±2	79±3 [a]
Hippocampus CA3	65±3	65±4	75±3
Hippocampus CA4	56±2	61±3	69±2 [a]
Dentate gyrus	68±2	74±4	83±3 [a]
Amygdala basolateral	74±3	93±4 [a]	93±5 [a]
Amygdala central	46±2	51±2	49±3
Caudate dorsolateral	102±4	113±4	119±6
Caudate dorsomedial	95±4	101±3	116±4 [a,b]
Caudate ventral	89±2	103±3 [a]	100±3 [a]
Sensory-motor cortex	92±4	129±4 [a]	111±5 [a,b]
Globus pallidus ventral	47±2	64±2 [a]	58±3 [a]
Globus pallidus dorsal	52±2	68±2 [a]	77±4 [a]
Entopeduncular nucleus	50±2	83±4 [a]	80±5 [a]
Subthalamic nucleus	83±4	146±4 [a]	120±8 [a,b]
Substantia nigra compacta	68±2	90±4 [a]	89±4 [a]
Substantia nigra reticulata	57±3	85±4 [a]	100±4 [a,b]
Ventral tegmental area	61±3	77±5	84±9
Thalamus antero-ventral	111±5	125±8	145±4 [a]
Thalamus ventro-medial	108±3	153±6 [a]	146±6 [a]
Thalamus ventro-lateral	89±4	115±6 [a]	112±6 [a]
Thalamus latero-dorsal	107±5	121±6	149±5 [a,b]
Thalamus medio-dorsal	104±5	146±6 [a]	150±7 [a]
Habenula medial	65±2	68±2	69±6
Habenula lateral (medial)	83±2	80±2	85±11
Habenula lateral (lateral)	102±4	81±2 [a]	87±9
Hypothalamus lateral	57±3	77±4 [a]	70±4
Medial geniculate body	112±4	120±3	126±4
Auditory cortex	123±1	135±5	149±8 [a]
Inferior colliculus	129±9	141±7	145±3
Lateral geniculate body	80±4	93±5	100±3 [a]
Visual cortex	89±5	98±4	106±6
Superior colliculus (external)	76±2	90±5	84±3
Superior colliculus (deep)	85±4	112±7 [a]	115±2 [a]
Cerebellar cortex	52±4	77±4 [a]	62±4 [a]

Values represent mean ± S.E.M. for the number of animals in parentheses. [a] $P < 0.05$ different from values in the control group, [b] $P < 0.05$ different from values in the apomorphine group, Bonferroni's t-test statistic.

3.2. Physiological parameters

Higher plasma glucose concentrations were measured in cocaine- and apomorphine-treated rats with respect to controls. Differences were not, however, statistically significant.

3.3. Local cerebral glucose utilization

Local rates of glucose utilization measured in the present study are summarized in Table 1. Briefly, repeated administration of either cocaine or apomorphine modified energy metabolism in portions of the extrapyramidal motor system, such as the globus pallidus, entopeduncular nucleus, substantia nigra, subthalamic nucleus, motor nuclei of the thalamus, and the lateral portion of the lateral habenular nucleus. However, the pattern of altered glucose utilization within the dopaminergic mesolimbic projection fields (nucleus accumbens and olfactory tubercle) differed between the two drugs. The effects of repeated administration of apomorphine were limited to the 'core' portion of the nucleus accumbens, where a 19%, statistically non-significant, increase in glucose utilization was measured. On the other hand, repeated administration of cocaine produced significant increases in glucose utilization in both the 'shell' and 'core' portions of the nucleus accumbens and in the olfactory tubercle. The effect of cocaine in the nucleus accumbens was stronger in the 'shell' portion than in the 'core'. Rates of glucose utilization in the 'shell' of cocaine-treated animals were significantly elevated in comparison to both controls and apomorphine-treated rats. Finally, repeated cocaine, but not apomorphine, altered energy metabolism in portions of the hippocampus.

4. Discussion

Increased behavioral response to repeated administration of dopamine receptor agonist drugs is a widely known process, although the mechanisms underlying this effect are not completely understood. The [^{14}C]2-deoxyglucose method, by mapping energy metabolism simultaneously in the entire central nervous system, allows to identify changes in cerebral function associated with specific pharmacological manipulations. Pre-

vious studies were performed to measure the effects of the acute administration of apomorphine (McCulloch et al., 1982) and cocaine (London et al., 1986; Porrino, 1993) on brain energy metabolism in the rat. Dose-dependent alterations of glucose utilization were measured in extrapyramidal motor areas of rats treated with either drugs. Furthermore, acute intravenous administration of cocaine and other psychomotor stimulants has been shown to increase glucose utilization in the mesolimbic dopaminergic areas, i.e. nucleus accumbens and olfactory tubercle (Porrino et al., 1984, 1988; Pontieri et al., 1990). On the other hand, acute intraperitoneal administration of cocaine failed to modify glucose utilization in the very same areas (London et al., 1986; Porrino, 1993). Based on those data, it has been hypothesized that the rapid modification of amine transmission obtained with the intravenous route of administration is necessary to sustain changes in glucose utilization at the level of the mesolimbic dopamine terminals. Moreover, the relevance of the route of administration has been emphasized in that psychostimulants are self-administered intravenously by experimental animals (Porrino, 1993). Here we demonstrate that repeated intraperitoneal administration of cocaine but not apomorphine produces increases in glucose utilization in the 'shell' of the nucleus accumbens and olfactory tubercle. The results of the present study provide, therefore, further evidence that changes in functional activity in the mesolimbic dopaminergic areas are a characteristic of psychomotor stimulant drugs and are not measurable with dopamine receptor agonists which do not bear psychostimulant properties. Therefore, the present data suggest that different functional regulations occur during the process of sensitization to psychostimulants with respect to non-psychostimulant dopamine receptor agonists. The effects of repeated intraperitoneal administration of cocaine are also different from those previously reported with acute intraperitoneal administration of cocaine (London et al., 1986; Porrino, 1993). The present data, therefore, demonstrate that the increased behavioral response produced by repeated intraperitoneal administration of cocaine is accompanied by changes in energy metabolism in the mesolimbic dopamine system, and suggest that altered functional activity may play a role in the process of sensitization.

The nucleus accumbens is a heterogeneous area where a 'shell' and 'core' can be identified based on a number of morphological and histochemical criteria (Heimer et al., 1991). Recently, we demonstrated that acute intravenous administration of cocaine and amphetamine, at dosages which sustain self-administration in the rat, increases glucose utilization preferentially in the 'shell' portion of the nucleus accumbens (Pontieri et al., 1994). Moreover, acute intravenous administration of self-administering dosages of cocaine, amphetamine, and morphine preferentially stimulates dopamine output in the 'shell' of the nucleus accumbens with respect to the 'core' (Pontieri and Di Chiara, manuscript in preparation). The behavioral effects of cocaine persist for a long time and involve changes in gene expression and protein synthesis. The relevance of the 'shell' in mediating these long-term effects was recently supported by the observation that acute administration of cocaine reduces rates of protein synthesis in most of the brain areas and, prominently, in the 'shell' (Orzi et al., 1995). Taken together, those previous data provide neurochemical evidence on a functional compartmentation within the nucleus accumbens and suggest that the 'shell', which represents the 'limbic' portion of the nucleus, is the most sensitive striatal locus of action of psychostimulants.

The results of the present study show that repeated, as well as acute, cocaine administration preferentially affects the 'shell' portion of the nucleus accumbens. These data also support the hypothesis that the 'shell' plays a key role not just in mediating the incentive motivational properties of cocaine but also in the process of motor sensitization to the drug.

Elevations in glucose metabolism were also measured in the hippocampal fields of cocaine-treated rats, although statistical significance was reached in CA2, CA4, and dentate gyrus only. It has been argued that the context in which drugs are administered plays a relevant role in the process of behavioral sensitization to psychostimulants. In the present study, sensitization was accomplished in the presence of a reinforcing context (i.e. the activity cage). In this respect, the increased functional activity within the hippocampus of cocaine-treated animals might be related to the environmental setting which potentiates the neurochemical 'memory' to the drug. Further studies will be necessary with the aim to separate the effects of the drug- and context-sensitization, and thus identify the relevance of the environment in producing the functional changes measured in the present study.

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