PHARMACOLOGY AND TOXICOLOGY

Self-Administration of Morphine by Rats Causes Monoamine Release in the Anterior Cingulate Cortex

S. K. Sudakov, I. V. Rusakova, M. M. Trigub, V. S. Kudrin*, and P. M. Klodt**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 8, pp. 177-180, August, 2007
Original article submitted April 27, 2007

Monoamine content in the microdialysate from the anterior cingulate cortex was measured in rats after injections and self-administration of morphine. Forced intraperitoneal injection of morphine did not lead to appreciable changes in the monoamine levels in the dialysate. Self-administration significantly increased monoamine levels in the extracellular space of the anterior cingulate cortex. Changes in catecholamine levels in the extracellular space of the anterior cingulate cortex correlated with the intensity of self-administration. The more morphine the animals injected to themselves, the greater was the increment in dopamine and norepinephrine levels. It seems that the increase of serotonin content in the anterior cingulate cortex did not depend on blood morphine concentration, but just reflected the fact of narcotic self-administration. The release of serotonin from nerve endings in the anterior cingulate cortex gradually increased at the beginning of the session, after which serotonin concentration started to decrease. The results indicate that monoamines are released from nerve endings in the anterior cingulate cortex only in response to self-administration of morphine. Catecholamines were released after each self-administration of the narcotic, while serotonin release seemed to be associated with the general status of the animal realizing this behavior.

Key Words: *life-time microdialysis; morphine self-administration; norepinephrine; dopamine; serotonin*

Psychoactive substance abuse is caused by their positive reinforcing effect. The subject using substances, causing dependence, experiences pleasant sensations, which forces him/her to repeat the dose. Positive support is a normal physiological mechanism, realized with participation of the endogenous reinforcement system [4]. Along with other brain structures, this system includes the ventral cegmental area, containing soma of dopamine neuron, locus coeruleus with noradrenergic neurons, and the central periaqueductal gray, containing neurons in which serotonin is synthesized [1]. Narcotic substances, including opiates, modulate neurons containing monoamines and promote the release of these neurotransmitters from nerve endings, located in the nucleus accumbens, prefrontal cortex, and

National Research Center of Narcology, Federal Agency for Health Care and Social Development; *V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences; **Research Institute of Human Morphology, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** s-sudakov@mail.ru. S. K. Sudakov

0007-4888/07/1442-0210

in the anterior cingulate cortex [8]. It was shown that morphine injection caused an increase of dopamine and serotonin concentrations in the interneuronal space of the nucleus accumbens [3]. It was found that morphine injections led to an increase in norepinephrine content in the cerebral prefrontal cortex (a critical state for manifestation of the positive reinforcing effect of the narcotic) [7]. Other authors [2] however, detected no increase in dopamine and norepinephrine levels in the prefrontal cortex after morphine injection. It was demonstrated that self-administration of heroin by rats, in contrast to forced injection, did not lead to significant changes in monoamine concentrations in the nucleus accumbens [6]. The release of dopamine, norepinephrine, and serotonin in the anterior cingulate cortex under the effects of opiates has never been described.

We measured monoamines in microdialysate from the rat anterior cingulate cortex after forced injection of morphine and after its self-administration by rats.

MATERIALS AND METHODS

Experiments were carried out on 20 male Wistar rats weighing 180-200 g at the beginning of the experiment. The rats were kept in cages, 10 per cage, at 21°C and artificial illumination (12:12 hours light:darkness) and free access to standard fodder and water.

Scalping of the skull, drilling of a hole, and stereotaxic insertion of a silicone-coated plastic CMA/12 cannula (CMA/Microdialysis) into the right anterior cingulate cortex by coordinates [5]: A +0.5; L 0.5; H 1.0 were carried out in all animals under general narcosis (ketamine 100 mg/kg and xylazine 100 mg/kg). After this the animals were implanted two-component synthetic catheters through the jugular vein. The intravenous part of the catheter was a 25 mm long silastic tube (Dow Corning Corp. outer diameter 1.2 mm). The tip of the catheter was placed in the initial fragment of the vena cava superior. The rest part of the catheter was a vinyl tube (Dural Plastic and Engineering) with the outer diameter of 1 mm, 55 mm long, connected to the intravenous portion through a special adapter (Small Parts Inc.), while its outer end was fixed to the skin on the animal neck on the dorsal side.

During the recovery period (3 days), the rats were kept in individual boxes with free access to water and fodder. After that they were placed into experimental boxes (Lafayette Instruments Inc.). A special device for microdialysis (CMA/Microdialysis) was inserted into the anterior cingulate cortex through the implanted cannula. The device consisted of a vinyl tube, insulated in steel coating, 0.64 mm in diameter. The tip of the tube (0.5 mm in diameter), protruding by 2 mm from the plastic cannula, was free from steel insulation and had characteristics of a semipermeable membrane with pores, through which molecules no larger than 20,000 Da penetrated into the tube from the perineuronal fluid. The adducing tip of the tube was connected to a precise pump (Harvard Apparatus) through a liquid rotating contact. The discharge tip of the tube was placed into a polypropylene tube for collection of microdialysate. The system was filled with artificial cerebrospinal fluid, pumped through the anterior cingulate cortex at a rate of 0.6 μl/min. After insertion of the microdialysis device into the anterior cingulate cortex the system was pumped through by artificial cerebrospinal fluid for 2 h for stabilization of neurochemical processes in the brain. Two control samples were then collected, each over 45 min. This was followed by intraperitoneal injection of 2 mg/kg morphine hydrochloride and collection of 2 more microdialysate samples, each over 45 min.

On the next day, the animals were placed into experimental boxes for morphine self-administration training. Free tip of the catheter implanted into the vein was connected (through a liquid rotating contact) to a pump filled with morphine solution. The rat was allowed to press the lever in the box, which led to injection of 100 μg morphine hydrochloride dissolved in 0.05 ml isotonic saline through the implanted catheter into the vena cava superior. The rats were placed into the experimental devices for studies of the intravenous morphine self-administration behavior for 5 days. During this period, 8 rats formed stable intravenous morphine self-administration behavior, when 1 injection (100 μg) of morphine was delivered after 3 lever-pressing acts (fixed ratio=3). After 2 days the rats were placed into experimental devices, the microdialysis and self-administration system was switched on, but no access to the lever was allowed. After insertion of the microdialysis device into the anterior cingulate cortex the system was pumped through with artificial cerebrospinal fluid until stabilization of monoamine levels in the brain. Two control samples were then collected over 20 min each. The access to the lever was then allowed for 1 h, during which the rat was allowed to press the lever and receive intravenous morphine unlimited. Three microdialysate samples were collected during this period (each over 20 min).

The samples were frozen at -70°C until the analysis. Defrosted samples were injected into high-

pressure liquid chromatograph for measuring dopamine, norepinephrine, and serotonin.

Monoamines in dialysates were separated and measured using an isocratic chromatographic system consisting of a Gilson 307 pump (Gilson) and LC-4B electrochemical detector (BAS). The components were separated in a HIPERSIL BDS C-18 analytical column (4 μ, 4.6×100 mm). In order to prevent destruction of catecholamine molecules in the light, a preserving agent (2 μl 0.4 M $HClO_4$) was added before collection of the sample. Norepinephrine, dopamine, and serotonin were measured at 23±1°C at the mobile phase rate of 0.5 ml/min and 170 bar pressure. The volume of the injector loop was 20 μl. The mobile phase: 0.1 M citrate-phosphate buffer containing 1.1 mM octansulfonic acid, 0.1 mM EDTA, and 9% acetonitrile (pH 3.0). The measurements were carried out on using carbon glass electrode at +0.85 V against Ag/AgCl reference electrode. Chromatograms were recorded and processed using the MultiChrome 1.5 software (Ampersand) analog-digital converter.

The results were statistically processed using Student's test for unpaired cases.

RESULTS

Basal concentration of dopamine was 33.3±6.2, norepinephrine 40.0±12.12, and serotonin 6.5±1.1 pmol/ml. Intraperitoneal injection of morphine did not appreciably change monoamine levels in the dialysate (Fig. 1).

Significant shifts in monoamine levels in the intercellular space of the anterior cingulate cortex were noted during self-administration of morphine. Dopamine content in the microdialysate collected during the first 20 min of self-administration was 2.5 times and that of norepinephrine 1.7 times higher than in the basal samples. Serotonin concentration in the anterior cingulate cortex during the first 20 min of morphine self-administration increased 3.4 times. During the next 20 min the concentrations of dopamine and norepinephrine virtually did not change, while serotonin level surpassed the basal value by 6.5 times. During the next 20 min, the content of dopamine in the anterior cingulate cortex increased by 50%, that of norepinephrine by 83%, while serotonin concentration started to decrease in comparison with the previous sample and only 2-fold surpassed the basal level (Fig. 2).

Experiments showed that the concentrations of free catecholamines (dopamine and norepinephrine) underwent uneven changes during morphine self-administration. The frequency of morphine self-administration was also irregular during the

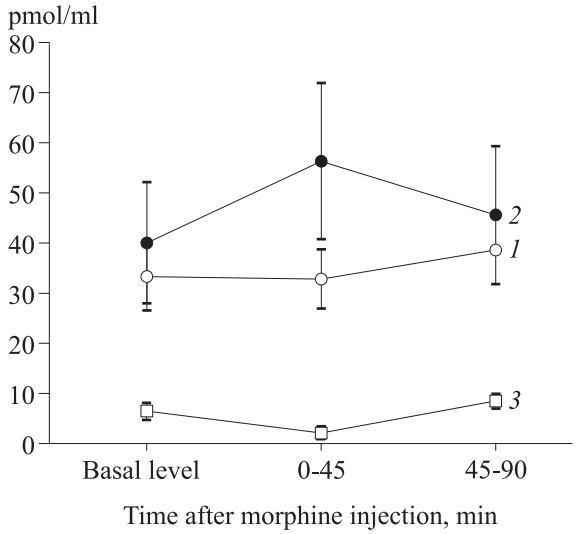

Fig. 1. Monoamine content in microdialysate collected from the anterior cingular cortex before and after intraperitoneal injection of morphine. Here and in Fig. 2: *1*) dopamine; *2*) norepinephrine; *3*) serotonin.

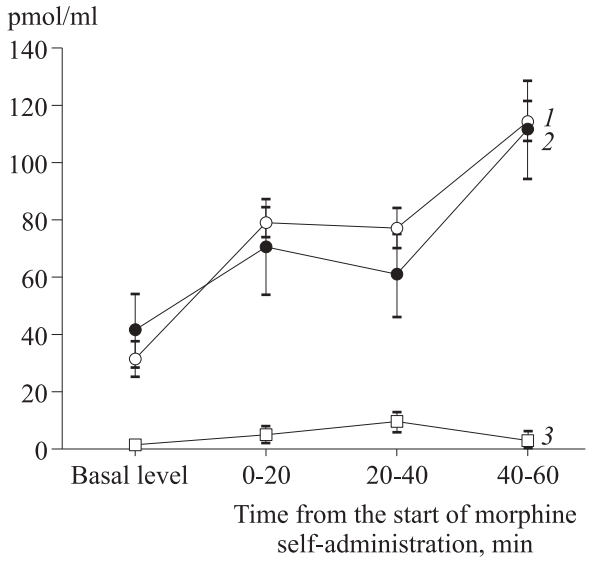

Fig. 2. Content of monoamines in the microdialysate collected from the anterior cingular cortex before and during intravenous self-administration of morphine.

session. The animals made 6.0±1.5 injections during the session and received approximately 3 mg/kg morphine. During the first 20 min the rats made 1.85±0.30, during the next 20 min 1.125± 0.400, and during the final 20 min 3.0±1.8 autoinfusions of morphine.

Hence, changes in catecholamine levels in the extracellular space of the anterior cingulate cortex corresponded to the intensity of self-administration. The more morphine the animal injected, the greater was the increment in dopamine and norepinephrine levels. It seems that the increase in

serotonin level in the anterior cingulate cortex did not depend on morphine concentration in animal blood, but just reflected the fact of the narcotic self-administration. At the start of the session, serotonin release from nerve endings in the anterior cingulate cortex gradually increased and then started to decrease.

These experiments showed that monoamine is released from nerve endings in the anterior cingulate cortex only as a result of self-administration of morphine. Catecholamines are released with each self-administration of the narcotic, while serotonin release seems to be associated with the general status of the animal, realizing this type of behavior.

REFERENCES

1. A. Dahlstrom and K. Fuxe, *Experientia*, **20**, No. 7, 389-399 (1964).
2. P. Devoto, G. Flore, L. Pira, *et al.*, *Psychopharmacology* (Berlin), **160**, No. 2, 220-224 (2002).
3. G. Di Chiara and A. Imperato, *Proc. Natl. Acad. Sci. USA*, **85**, No. 14, 5274-5278 (1988).
4. G. F. Koob, A. J. Roberts, G. Schulteis, *et al.*, *Alcohol. Clin. Exp. Res.*, **22**, No. 1, 3-8 (1998).
5. G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates*, London (1986).
6. J. E. Smith, C. Co, M. D. Coller, *et al.*, *Neuropsychopharmacology*, **31**, No. 1, 139-150 (2006).
7. R. Ventura, A. Alcaro, and S. Puglisi-Allegra, *Cereb. Cortex*, **15**, No. 12, 1877-1886 (2005).
8. J. Vetulani, *Pol. J. Pharmacol.*, **53**, No. 4, 303-317 (2001).