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THE EFFECTS OF RUNNING SPEED AND RUNNING DURATION ON EXTRACELLULAR DOPAMINE LEVELS IN RAT STRIATUM, MEASURED WITH MICRODIALYSIS

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ABSTRACT

Dopamine (DA) has an important role in the control of motor behaviour. The **purpose** of this study was to examine the effects of different exercise protocols on extracellular DA levels in rat striatum.

Methods: The microdialysis technique was used to collect extracellular concentrations of DA from the striatum of twenty-six male albino Wistar rats. One of the following protocols was used: '12-20': running at 12m/min during 20 min; '12-60': running at 12m/min during 60 min; '26-20': running at 26m/min during 20 min; '26-60': running at 26m/min during 60 min.

Results: In all groups extracellular DA levels significantly increased during running and during recovery from exercise. With an exercise duration of 60 min extracellular DA levels stayed significantly elevated until the end of the experiment in both groups (128.3% low speed; 153.3% high speed respectively). Although the 20 min. duration groups showed no significant difference between high and low speed, extracellular DA levels rose faster and stayed relatively higher in the high speed group. 3-Factor ANOVA (speed x duration x time) showed no significance for the speed factor ($p = 0.19$), while exercise duration and time were both significant ($p = 0.006$ and $p = 0.00006$ respectively). No interaction effect was found between speed and duration ($p = 0.74$), speed and time ($p = 0.82$) and speed, duration and time ($p = 0.97$). There was a significant interaction effect between exercise duration and time ($p = 0.00017$).

Conclusion: the present results demonstrate that extracellular DA levels are more likely to be linked to the exercise duration than to exercise speed, and that the perturbation of extracellular DA levels lasts for a longer time period when exercise duration is longer.

Key words: neurotransmission, dopamine release, brain

Introduction

Research into the physiological effects of exercise usually ends at the muscular or neuromuscular level even though it is apparent that there is also an influence on the central nervous system, with convincing evidence that several neurotransmitters are involved in control of locomotion (13, 16, 21, 22, 23, 36). The basal ganglia consist of 5 subcortical nuclei that are interconnected and functionally related and participate in the control of locomotion, together with the cerebellum, the corticospinal tract and the brain stem motor nuclei. Al-

though there are dopamine-containing neurons scattered throughout the central nervous system, most of the neurons originate from the substantia nigra and the ventral tegmental area in the midbrain (2). The projections to the striatum facilitate the initiation of voluntary movements, and one important role of striatal dopamine (DA) is to work in the control of motor function (18).

A number of different functions have been ascribed to the mesocorticolimbic dopamine system. The nigrostriatal dopaminergic system is a motor-sensorimotor integration area within the brain that has been associated with the pathology of Parkinson's disease (10). Dopamine has an important role in the control of motor behavior. In patients with Parkinson's disease, loss of dopaminergic neurons leads to dopamine depletion in the brain and drastically reduced voluntary movement (2). The relationship between the nigro-striatal dopaminergic system and motor behavior has been substantiated by results from numerous experimental paradigms utilizing animal models (10). DA depletion in Parkinsonian patients or Parkinson models in animals (6-OHDA lesioned animals) induces various kinds of motor dysfunction i.e. reduced voluntary movement, retardation of motor initiation, impairment of skilled task, abnormal posture, and locomotion disorders (5, 6, 30, 32, 35). Running or physical exercise represents a physiological challenge for the organism, which is characterised by a perturbation of homeostasis. A number of studies have examined the effects of exercise on several neurotransmitters (7, 12, 25). Acute treadmill running is reported to result in no change (15) or to increase the brain levels or metabolism of DA and 5-HT (4, 8, 9, 13, 19). Treadmill locomotion, thus influences neurotransmitter levels in several brain areas, but it is unknown whether this physiological stimulus leads to a change in neurotransmitter release, since all these earlier studies were performed on homogenised tissue. Microdialysis is a method to assess alterations in neurotransmitter levels in brain extracellular space (27). This method allows the measurement of local neurotransmitter release in combination with ongoing behavioral changes such as exercise. Our recent microdialysis studies already indicated that exercise increases neurotransmitter release in different brain area's (24, 26) and that exercise training decreases baseline striatal neurotransmitter output (28). However, it is unknown whether a difference in intensity and duration of this physiological stimulus leads to a difference in neurotransmitter release in rat striatum.

Therefore, the purpose of this study is to examine the effects of different exercise protocols on extracellular DA levels in rat striatum, using the *'in vivo'* microdialysis sampling technique.

Materials and Methods

The protocols for the animal experiments described in this paper were carried out according to the national rules on animal experiments and were approved by the Ethics Committee of the Faculty of Medicine and Pharmacy of the Vrije Universiteit Brussel.

Animals

Twenty-six male albino Wistar rats (200-300g) were used. The animals were kept on a 12 h light/dark cycle (lights on at 6 a.m.) and were fed a standard diet with free access to food and water throughout the experiment. To minimise the effects of circadian variations in brain neurotransmitters, all experiments started at 9 a.m.

The animals were randomly assigned to one of the 'speed' or 'duration' groups. In this way four groups of animals could be

compared:

(i): '12-20': running at 12m/min during 20 min
(ii): '12-60': running at 12m/min during 60 min
(iii): '26-20': running at 26m/min during 20 min
(iv): '26-60': running at 26m/min during 60 min

Two weeks before the experiment, the animals were placed on the treadmill twice a week in order to let them adapt to the experimental situation. The animals of the 2 'low-speed' groups exercised for 15 or 45 min at 12m/min respectively, while the animals of the 'high-speed' groups exercised for 15 to 45 min at 26m/min respectively to become familiarized with running. In the final adaptation session animals ran at the same speed and duration as on the experimental day. Four animals were reluctant to run during this testing period, and were replaced by others. All animals had their last 'adaptation session' 3 days before surgery.

Surgery and brain dialysis

Animals were anaesthetised with a mixture of ketamine and diazepam (50 mg/kg: 5 mg/kg i.p.) and placed on a stereotaxic frame. The skull was exposed and a guide cannula (CMA Microdialysis, Stockholm, Sweden) was implanted through a bore hole in the left striatum (A: +1.2, L: -2.4, V: +2.8) according to Paxinos & Watson (29). The animals were allowed to recover from surgery for two days, then the microdialysis probe (CMA12, membrane length: 3 mm) (CMA Microdialysis, Stockholm Sweden) was inserted. The probes were connected to a microinfusion pump and perfused with a modified Ringer's solution (147.5 mmol/l Na^+, 4 mmol/l K^+, 1.1 mmol/l Ca^{2+}, 153.7 mmol/l Cl^-) at a constant flow rate of 2 µl/min. After the microdialysis probe was implanted, the animals were placed on the treadmill and they remained there until the end of the experiment. The sampling of the dialysates began 20 hours after probe implantation. The microdialysis samples were collected every 20 minutes in vials containing 10µl of an anti-oxidant mixture (0.01M HCl; 0.1% $Na_2S_2O_5$; 0.01% Na_2EDTA). The day of the experiment samples were collected for at least two hours (six samples) to verify stable basal conditions. Then the animal exercised and sampling continued during recovery from exercise, total collection time being at least 300 min.

Exercise

The day of the experiment, when stable basal dialysate neurotransmitter levels were reached, the animals were exercised on a motor driven horizontal treadmill (Omnitech electronics, Columbus, Ohio, USA). An adjustment was made to the treadmill in order to attach the counter-balance arm of the microdialysis system. One of the following protocols was used: '12-20': running at 12m/min during 20 min; '12-60': running at 12m/min during 60 min; '26-20': running at 26m/min during 20 min; '26-60': running at 26m/min during 60 min.

Analytical assay

Microbore LC with electrochemical detection was used for the determination of DA in the obtained dialysates as described previously (28). The LC system consisted of a «Gilson 302 pump» (Gilson, Villiers le Bel, France) operating at a flow rate of 0.7 ml/min with a flow splitter kit for SepStikâ microbore colums (Bioanalytical Systems, West Lafayette, IN, USA) resulting in a flow rate through the column of 58 µl/min. Separation was performed on a SepStikâ microbore column: 100mm x 1 mm i.d., 5 µm C8 (Bioanalytical Systems, West Lafayette, IN, USA), which was

coupled to a Kontron autosampler (Kontron instruments, Milan, Italy). The injection volume was 10 µl. The electrochemical detector used was a single channel amperometric capillary detector (Antec, Leiden, The Netherlands), based on the wall-jet principle. The cell was equipped with a glassy carbon working electrode. The operating potential was 450 mV versus a Ag/AgCl reference electrode. The range of the detector was set at 0.2 nA/V. Integration of the chromatograms was performed with an integration computer program (Kontron Data System, 450-MT2, Kontron, Milan, Italy).

The mobile phase consisted of 60 mM sodium acetate, 2 mM decanesulphonic acid and 0.1 mM Na_2EDTA. To 200 ml of this solution 25 ml acetonitrite is added.

Data analysis

Data are given as mean ± SEM. The extracellular DA levels in the dialysates are expressed in fmol/20min and not corrected for relative recovery across the dialysis membrane. The levels obtained during and after the exercise are expressed as percentages of the baseline value. The baseline value is the average concentration of the neurotransmitter in the dialysates obtained during the first 2 hours of dialysis. All subsequent values are expressed relative this basal values (=100%). To examine the possible influence of exercise speed and/or duration a 3 factor ANOVA (speed x duration x time) was used followed by Fischer PLSD-test ($p = 0.05$).

Results

Baseline DA concentrations were comparable in all groups (183.0 ± 21.1 fmol/20min). In all groups extracellular DA levels significantly increased during running and during recovery from exercise. With an exercise duration of 60 min (groups 12-60 and 26-60) extracellular DA levels stayed significantly elevated until the end of the experiment in both groups (128.3% low speed; 153.3% high speed respectively).

Although in the 20 min duration groups (groups 12-20 and 26-20) there was no significant difference between high and low speed, extracellular DA levels rose faster and stayed relatively higher in the high speed group (see figure 1).

3-Factor ANOVA (speed x duration x time) showed no significance for the speed factor ($p = 0.19$), while exercise duration and time were both significant ($p = 0.006$ and $p = 0.00006$ respectively). No interaction effect was found between speed and duration ($p= 0.74$), speed and time ($p= 0.82$) and speed, duration and time ($p= 0.97$). There was a significant interaction effect between exercise duration and time ($p= 0.00017$).

Discussion

The present data show that during exercise extracellular DA levels in striatum are more influenced by the duration than the speed of exercise. Indeed, when animals are running at the same speed, the extracellular levels significantly differed between the 20 min and 60 min protocol. However, it should be noted that this was only so from min 80 i.e. sixty minutes after the short duration exercise group finished running, or in the first post-exercise sample of the long duration exercise group.

The data of our study confirm the results of previous experiments. Hattori et al. (17, 18) found similar increases in striatal DA during 20 minutes of exercise on a treadmill at a moderate speed (18m/min). Taken together their findings indicate that from the moment an animal exercises at a speed faster than walking (i.e. about 8 to 10m/min), striatal extracellular DA levels increase significantly.

This increase in the striatum is compatible with the currently held view that the

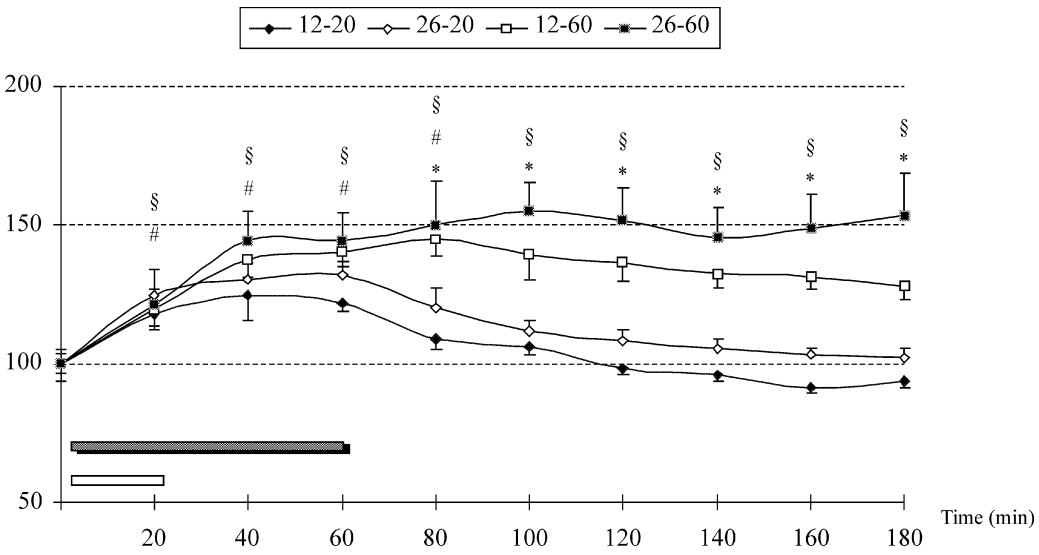

Figure 1: *Extracellular DA levels in rat striatum during running (20 or 60 min at two different speeds) and recovery of exercise*
Bars indicate running duration: open bar = 20 min of exercise; solid bar = 60 min of exercise.

Extracellular DA levels increased significantly in all groups during running and during recovery from exercise (i.e. until min 80 for the short duration groups, and until the end of the experiment for the long duration groups).
significant increase from baseline for the short duration exercise group ($p<0.05$)
§ significant increase from baseline for the long duration exercise ($p<0.05$)
* significant difference between the two 60 min duration and 20 min duration groups ($p<0.05$).

nigrostriatal dopaminergic neurons are associated with motor function. It is however, rather difficult to compare the results of the few studies that performed microdialysis in rat striatum during exercise since not all experimental protocols are similar. Some studies that tried to use a straight or circular treadmill sometimes used other stimuli together with exercise.

The study of Freed & Yamamoto (13) already indicated that the neurophysiological action of DA in movement control is brain region specific. The motor nuclei receiving dopaminergic input in striatum have a functional specificity i.e. the nucleus accumbens is strongly involved in the speed of movement, while the caudate nucleus is more involved in posture. Freed & Yamamoto (13) further showed that in both brain areas (caudate and accumbens) the concentrations of DA and DOPAC increased progressively with speed, but that the DA concentrations in the n. accumbens correlated more with the speed than the DA concentrations in the n. caudatus. However, in this homogenate study it was not fully clear if the extracellular levels (i.e. the release) of DA increased and one has to consider that other variables could also influenced the results, since the animals walked and ran on a straight treadmill after water deprivation. During the experiment, a water dropper was provided in front of the animals in order to keep them oriented in a forward-moving direction. This reinforcement could have influenced the results, since it is known that DA plays a modulatory role in thirst regulation (11, 37). Furthermore other similar experiments (32, 33) have shown that drinking also significantly increased DA.

Since then some studies have tried to reproduce similar experiments, but there were controversies in the subsequent stu-

dies using variations of this model, which showed that neither DA nor DOPAC increased (34), or just an increase of the metabolites (32, 33) was found by conditioned circling. Bertolucci-D'Angio et al. (3) used in vivo voltammetry to study the DA metabolism in different brain regions and compared forced locomotion with several other stressors. Forced locomotion of rats on a rotarod for 40 minutes increased the amplitude of the DOPAC oxidation peak in the striatum and the nucleus accumbens, but failed to affect the DOPAC peak in the prefrontal cortex. The study of Freed & Yamamoto (13) used low speeds (1.44 to 7.2 m/min) of movement and they discovered increased brain DA and DOPAC concentrations, while more recent microdialysis studies also using low speed movements did not find increases in striatal DA (6, 18). This last study found that the threshold speed of running for the increase of DA, DOPAC and HVA release was around 6.6 m/min. They further found that once this threshold was passed, DA increase was not influenced by the running speed. In another experiment, these authors (17) had already registered similar increases in extracellular DA during 20 min of exercise at 18m/min as found in our experiments (26).

The results of the present study together with those of Hattori et al. (17, 18) indicate that from the moment an animal exercises at a speed higher than 'walking speed', the duration of exercise plays a more dominant role than the speed of running.

On the other hand, extracellular DA levels reflect the net balance between release and uptake of the neurotransmitter in the synapse, and it seems that when the disturbance brought about by exercise continues, the extracellular DA concentration, levels off at about 50% above baseline. This increased extracellular concentration continues even when exercise is stopped. It is however not possible with this experimental set-up to determine if the higher levels of extracellular DA in the long duration exercise-groups are brought about by net release, changes in reuptake, or a combination of these factors.

When discussing the role of DA in behaviour, the contribution of the different dopaminergic pathways are often simplified to motoric and emotional components. The striatum is believed to play a role in motoric functions, whereas the limbic and cortical neurons are linked to motivational and cognitive components of behavior (14). Various workers have correlated locomotion and DA levels in dialysates (17, 18, 30, 31). Most studies seem to agree that (as in other behaviors) DA release is only slightly (but most of the times significantly) elevated due to exercise. In contrast, emotional, aversive and rewarding stimuli clearly induced quantitative differences in the various dopaminergic regions (1, 3, 20).

In conclusion the present results demonstrate that extracellular DA levels are more likely to be linked to the exercise duration than to exercise speed, and that the perturbation of extracellular DA levels lasts for a longer time period when exercise duration is longer.

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