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Endurance training effects on striatal D_2 dopamine receptor binding and striatal dopamine metabolite levels

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We have previously shown that endurance training is associated with higher binding of [^{3}H]spiperone to striatal D_2 dopamine (DA) receptors of presenescent (21 months old) rats. In the present study we investigated the effects of 6 months of endurance training of young adults on the relationship between steady-state levels of DA and its metabolites in striatum and the affinity and density of striatal D_2 DA receptors. The extent of training was confirmed by evaluating the maximal oxygen consumption ($\dot{V}O_2$ max) in the subjects. D_2 DA binding was significantly increased at each of 3 [^{3}H]spiperone concentrations in the young runners. A 'synaptic coupling ratio' calculated as the specific DA binding/DOPAC concentration was significantly increased in runners for the 0.1 and 0.4 nM radioligand concentrations. Across experimental groups levels of DA were highly and positively correlated with specific DA binding at the 0.1, 0.2 and 0.4 nM [^{3}H]spiperone concentrations. Together, these results suggest that exercise can alter the number of DA binding sites and the metabolism of DA in young adult animals.

It has been suggested that the benefits of exercise occur through actions on many organ systems [1, 6, 15] and that the role played by brain systems in the adaptive response to exercise may be amplified by aging [16]. The extent to which endurance training can modify characteristics of nigrostriatal dopamine (DA) neurons and of striatal DA receptors is an area with both theoretical and practical importance. Yet, the relationships between chronic endurance training and central nervous system transmitter functions have not been examined in any detail. Thirty minutes of exercise in untrained animals decreased DA levels in hypothalamus, striatum, cortex and

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midbrain [13]. We observed that increased D_2 DA receptor binding in striatum was correlated with enhanced skeletal muscle oxidative capacity following 12 weeks of treadmill conditioning of young adult Sprague–Dawley rats [8], and that endurance training of older Sprague–Dawley rats (18 months of age at the initiation of training) appeared to protect striatal DA metabolite levels from age-associated increases and D_2 DA receptors from age-associated decreases [14]. Portions of our original findings have been confirmed using a complementary training technique [5]. In the present study, we determined the effects of 6 months of endurance training on the relationships among steady-state, in vivo levels of DA and its metabolites and the in vitro affinity, and density of striatal D_2 DA receptors.

Male Sprague–Dawley rats were purchased from Harlan–Sprague–Dawley (Indianapolis, IN). Animals were held in the Animal Resources Center of the University of Texas at Austin throughout the experiment. Animals were single housed in clear Plexiglas cages with ad libitum access to food and water throughout the experiment. A 12-h light–dark cycle (lights on 7.00–19.00 h) was maintained during the experiments with training and sacrifice conducted during the period from 9.00 to 12.00 h. Endurance training was conducted as previously described [8, 14]. Peak oxygen consumption was determined by the method of Brooks and White [3]. Peak oxygen consumption was determined during the final month by monitoring oxygen consumption during a progressive treadmill test performed on an 18° incline, which elicits the highest treadmill oxygen consumption in rats. Within one month of peak oxygen consumption measurement, approximately half of the animals in each group performed submaximal treadmill run tests. Oxygen consumption and respiratory exchange ratio were measured using the metabolic chamber used for peak oxygen consumption measurement.

[^{3}H]spiperone ([^{3}H]SP; 27.6 Ci/mol), was purchased from New England Nuclear (Boston, MA). Ketanserin was donated by Janssen (New Brunswick, NJ). (+)-Butaclamol was purchased from Research Biochemical International (Wayland, MA). Dihydroxybenzylamine hydrobromide (DHBA) was purchased from Sigma. Following sacrifice, the striata were rapidly dissected over ice, placed in 1000 μl of 50 mM Tris buffer (pH 7.7) and homogenized at the lowest possible speed with a Brinkman Polytron. A 200-μl portion was placed in a microcentrifuge tube containing 160 μl of 10% trichloroacetic acid and 2 ng of an internal standard, DHBA. This was placed on dry-ice and stored at -80°C, in preparation for determination of DA, dihydrophenylacetic acid (DOPAC), and homovanillic acid (HVA) concentrations. The remaining 800 μl were stored at -80°C for [^{3}H]SP receptor binding.

For binding studies, the homogenate was thawed on ice, diluted with 50 mM Tris buffer (pH 7.1) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$, and homogenized. After centrifugation (50,000 g) for 15 min, the pellet was resuspended in 50 mM Tris buffer (pH 7.1) and homogenized. The tissue preparation yielded a protein concentration of approximately 80 μg/ml in a 2-ml total incubation volume. Duplicate incubation tubes contained the following: Tris buffer (pH 7.1), [^{3}H]SP (0.1, 0.2, 0.4 or 0.8 nM; $K_d = 0.13$ nM) and tissue. Tubes for the non-specific ligand binding contained the same as above plus 1 μM (+)-butaclamol as blank.

Ketanserin, at a concentration 200-fold that of the [^{3}H]SP, was added to all tubes to mask the serotonergic component of the binding [9]. Incubations (15 min at 37°C) were terminated by rapid filtration under vacuum (1 ml/s) through Whatman GF/B filters. Filters were rinsed 3 times with 5 ml of ice-cold Tris buffer (pH 7.7), placed in 10 ml liquid scintillation cocktail (HP Ready-Solv: Beckman) and counted 24 h later by liquid scintillation spectrometry (efficiency = 45%). Saturation binding data were analyzed for each animal [11, 14].

For metabolite determinations, the homogenate was thawed on ice, centrifuged at 20,000 *g* for 30 min, and the supernatant filtered (Bioanalytical Systems MF-1, 0.45 micron regenerated cellulose filters). Sixty microliters of filtrate were injected for chromatographic analysis of DA, DOPAC and HVA. The chromatographic system consisted of a Beckman model 112A pump, model 210 injector value (20 μl loop) and a reverse phase 3 micron ultrasphere C18 analytic column (0.46 × 15 cm) protected by a 4 cm prefilter containing 10 micron packing. Column eluant was monitored by a Bioanalytical Systems amperometric detector model LC4A with detector potential set at +0.65 V using a glassy carbon working electrode. The LC4A detector (sensitivity set at 1 nA full scale) was connected to a Shimadzu CR1A data processor for initial peak processing. The mobile phase consisted of 0.1 M NaH_2PO_4, 250 mg/l sodium ethylenediamine tetraacetic acid (Na_2EDTA), 60 mg/l sodium octyl sulfate (SOS; ion pairing reagent) and 2% reagent grade methanol. With a 1.2 ml/min flow rate typical retention times were: DHBA = 3.9 min, DOPAC = 4.7 min, DA = 7.7 min, and HVA = 12.6 min. Protein content was determined by the method of Lowry et al. [12]. Data from the $\dot{V}O_2$max, D_2 DA binding, and DA metabolite levels determinations were analysed by ANOVA followed by post-hoc Newman–Keuls tests [19].

The $\dot{V}O_2$max of young trained animals was enhanced relative to that of sedentary rats (90 ± 3 vs 76 ± 3 ml/(kg·min), $P < 0.05$). The treadmill speeds used to elicit these

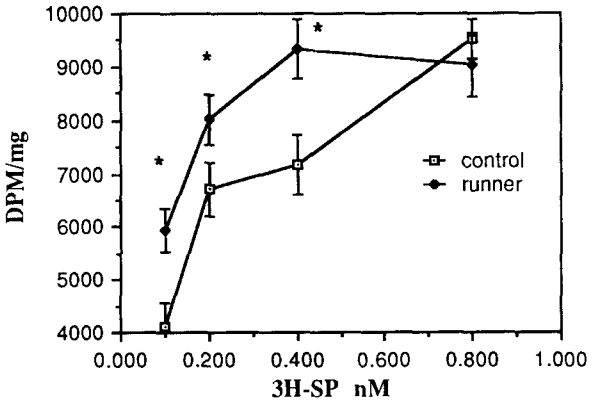

Fig. 1. Exercise effects on striatal D_2 DA receptors in young adult (9 months old) Sprague–Dawley rats. Rats were sedentary (control) or endurance trained on a treadmill for 6 months (runner). D_2 DA receptor binding was evaluated as described in the text with [^{3}H]SP using (+)-butaclamol as a blank and ketanserin as a mask for serotonin-2 sites.

TABLE I

EFFECTS OF ENDURANCE TRAINING ON STRIATAL DA METABOLITES AND D_2 DA RECEPTORS IN YOUNG ADULT SPRAGUE–DAWLEY RATS: CORRELATIONAL ANALYSES[a]

Group	Variables	Correlation	Significance
(A) Combined sample ($n=30$)	DA w/Bound (0.1 nM)	0.79	0.001
	w/Bound (0.2 nM)	0.53	0.001
	w/Bound (0.4 nM)	0.94	0.001
	w/B_{max}	−0.16	n.s.
(B) Full sample[b]			
Young control ($n=15$)	DOPAC w/HVA	0.84	0.001
	DA w/Bound (0.1 nM)	0.80	0.001
	w/Bound (0.2 nM)	0.75	0.001
	w/Bound (0.4 nM)	0.99	0.001
	w/B_{max}	0.04	n.s.
Young runner ($n=15$)	DOPAC w/HVA	0.43	0.056
	DA w/Bound (0.1 nM)	0.72	0.001
	w/Bound (0.2 nM)	0.17	n.s.
	w/Bound (0.4 nM)	0.92	0.001
	w/B_{max}	−0.52	0.024
(C) Subset of full sample[c]			
Young control ($n=7$)	DOPAC w/HVA	0.75	0.027
	w/DA	0.85	0.013
	DA w/Bound (0.1 nM)	−0.27	n.s.
	w/Bound (0.2 nM)	−0.10	n.s.
	w/Bound (0.4 nM)	−0.06	n.s.
	w/B_{max}	−0.28	n.s.
Young runner ($n=10$)	DOPAC w/HVA	0.75	0.006
	w/DA	0.81	0.001
	DA w/Bound (0.1 nM)	−0.16	n.s.
	w/Bound (0.2 nM)	−0.12	n.s.
	w/Bound (0.4 nM)	−0.35	n.s.
	w/B_{max}	−0.28	n.s.

[a]Levels of DA and its metabolites were as follows: young runners: DOPAC = 9.3 ± 0.5, HVA = 6.8 ± 0.3, DA = 126 ± 4 ng/mg; young controls: DOPAC = 10.2 ± 0.7, HVA = 7.3 ± 0.6, DA = 122 ± 9 ng/mg. Calculation of the ratio of [^{3}H]SP binding at 0.1, 0.2 and 0.4 nM to DOPAC or HVA in each subject yielded the following significant changes: (1) 0.1 nM [^{3}H]SP/DOPAC: young runner = 653 ± 59, young control = 466 ± 60 DPM/ng; (2) 0.4 nM [^{3}H]SP/DOPAC: young runner = 1030 ± 86, young control = 782 ± 63 DPM/ng.

[b]All subjects in both experimental and control groups provided data for the correlational analyses.

[c]Subsets of each group were derived based on affinity of binding (criterion for inclusion was $K_d < 99$ pM).

training effects were 42.6 ± 1.8 m/min (trained) and 32.4 ± 1.0 m/min (untrained; $P < 0.05$). These results indicate a training effect induced by the endurance training regime which is consistent with that reported in our previous studies [7, 8, 14] and in the literature [4].

The specific striatal binding of [^{3}H]SP to D_2 DA receptors at 0.1, 0.2 and 0.4 nM was increased by endurance training (Fig. 1). These results replicate and extend a previous finding from our laboratories [8]. However, the maximal receptor density (B_{max}) of the endurance training group was not different from that of the sedentary group. The binding affinities (K_d) also were not significantly different from one another (at 111 pM estimated from 4-point Scatchard analyses with protein concentrations of 175 μg/assay; ANOVA). Endurance training had no effect on steady-state levels of DA or its major metabolites in striatum (no significant changes from DOPAC $= 10.2 \pm 0.7$, HVA $= 7.3 \pm 0.6$, and DA $= 122 \pm 9$ ng/mg prot.; Table I). Similary, the ratios of the metabolites to DA were also not altered in the endurance trained group. However, since binding and DA metabolite data were obtained from the same tissue in the same animal it was feasible to attempt to estimate the degree of 'synaptic coupling' in these animals as the ratio of the binding to the levels of DOPAC and HVA, respectively. As indicated below, it appeared reasonable to calculate the ratios as specific D_2 DA binding at each of the 3 lower radioligand concentrations vs metabolites. The ratios were significantly increased for D_2 DA binding/DOPAC at two of the 3 [^{3}H]SP concentrations.

Because both levels of DA and its metabolites and D_2 DA binding values were available from each animal with a sufficiently large number of subjects to allow correlational analyses, it was of interest to determine if relationships between the neuronal and receptor markers existed. Table I presents the results of these analyses. Across the combined control and exercise groups there was a striking positive correlation between levels of DA and the specific D_2 DA binding at 3 of the 4 radioligand concentrations and these high correlations were maintained in general when within-group correlations were examined. In order to establish a putative basis for these correlations, it was decided to evaluate a subset of each group which exhibited a somewhat higher affinity of binding to D_2 DA sites. Data were selected blind from each group if the K_d was < 99 pM. This resulted in a sample of 10 runners and 7 controls. The K_d in these animals was the same for the two groups (54 ± 0.5 pM) and the B_{max} showed the same relationship as in the full groups (404 ± 32 vs 356 ± 34 fmol/mg prot. for runners and controls, respectively). As indicated in the table, in these two groups the correlations between DOPAC and HVA, and between DOPAC and DA were consistent and strikingly positive, but the relationship between DA and the D_2 DA binding disappeared.

The major finding of the present report is that 6 months of endurance training alters neurochemical markers of the nigrostriatal DA system in young adult rats. Specifically, binding of [^{3}H]SP to D_2 DA receptors and the ratio of binding to levels of DOPAC were enhanced in the runners. Together, these results suggest that a shift in DA function may occur as a result of exercise, either due to altered DA release or to changes in D_2 DA binding sites. These results complement and extend our pre-

vious findings of the effects of endurance training on striatal DA metabolites and D_2 DA receptors in presenescent, older rodents [14]. In that report [^{3}H]SP binding at each of 4 concentrations was significantly higher in the runners than in the controls as was the B_{max} for binding to D_2 DA sites. In addition, the age-associated increases in DA metabolites which appeared in the presenescent sedentary animals did not occur in the runners. Thus, those results suggested that endurance training was able to induce an apparent stabilization of more youthful values for striatal DA neuronal and receptor markers compared to values obtained in sedentary controls. Taken together with the present results, these data suggest that endurance training has significant actions throughout the lifespan to enhance D_2 DA binding and to reduce the synaptic content of DA, although the effect of training on these parameters appears to increase with the age of the animal. In a previous study [14], DOPAC- and HVA-to-DA ratios were significantly lower in the 24-month old runners than in the age-matched controls, suggesting an ability of endurance training to stabilize metabolite levels at values consistent with those in the young adult. A similar trend was noted in 9-month old rats in the present study. These results suggest that endurance training alters the synaptic relationship between levels of metabolized striatal DA and D_2 DA receptor numbers in a reciprocal manner.

A particularly striking finding in the present report was the high positive correlation between levels of striatal DA and D_2 DA binding. The lack of a similar relationship between DA content and the binding at the 0.8 nM radioligand concentration and at the B_{max} suggested to us that these latter two values might not be as descriptive of the underlying physiology as the binding at the 3 lower radioligand concentrations. This is consistent with the observation that the binding had saturated by 0.2 nM in the runners (Fig. 1). It also suggested that it might be useful to examine separately the relationships between neuronal and receptor markers in a subset of the total population which exhibited a higher binding affinity. The finding of a putatively different pattern of correlations among neuronal markers and between neuronal and receptor markers in the high- and low-affinity groups suggests that endurance training may have differential actions depending on the initial neurochemistry of the individual. Certainly, such a hypothesis must be tested directly and studies are planned with this in mind.

Our studies of endurance training effects on DA metabolite levels vs DA binding in striatum suggest that a reciprocal pattern of change may be detected after exercise. Normal age effects in endurance trained rats appeared to be damped in amplitude relative to those in untrained animals. The study of exercise effects on brain transmitters will probably need to include evaluations of transmitter interactions. This becomes most feasible when both synaptic content and receptor binding of each transmitter are measured. Such experiments can extend present knowledge by showing exercise effects as putative patterns among components of functional sensorimotor circuits.

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