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Changes in paroxetine binding in the cerebral cortex of polydipsic rats

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Abstract

Schedule-induced polydipsia was induced when food-deprived rats were subjected to a fixed-time (60 s) feeding schedule for 150 min daily for 3 weeks (training period). Subsequent chronic administration of the serotonin reuptake inhibitor fluoxetine reduces schedule-induced polydipsia over 2–4 weeks. We asked whether changes in the serotonin reuptake carrier occur following the development of schedule-induced polydipsia and its reduction by fluoxetine. Using [^{3}H]paroxetine binding, we found a 40% increase in K_d and a 50% decrease in B_{max} in polydipsic rats; both were reversed by fluoxetine. Food deprivation alone did not affect these parameters. These observations suggest that changes in the serotonin reuptake carrier correlate with the development and reversal of schedule-induced polydipsia.

Keywords: Reinforcement schedule; Drinking behavior; 5-HT (5-hydroxytryptamine, serotonin) uptake inhibitor; Obsessive-compulsive disorder

1. Introduction

Schedule-induced polydipsia is a phenomenon belonging to a more general class of behaviors termed 'adjunctive' (Falk, 1971; Pellon and Blackman, 1992; Woods et al., 1993). Schedule-induced polydipsia is produced in food-deprived rats subjected to a procedure in which food is delivered with a fixed-time feeding schedule of between 60 and 180 s (Falk, 1971). These animals have been shown to drink unusually large amounts of water if given the opportunity to do so. Adjunctive behaviors have been cited as potential animal models for human compulsive disorders (Pitman, 1989) such as obsessive compulsive disorder (Woods et al., 1993). Since obsessive compulsive disorder and schedule-induced polydipsia both involve excessive expression of a normal behavior, the polydipsia model may be useful for the prediction of compounds that are effective in the treatment of obsessive compulsive disorder (Woods et al., 1993).

Chronic administration of the selective serotonin reuptake inhibitors fluoxetine, clomipramine and fluvoxamine has been found to significantly reduce polydipsia after 2 weeks and throughout the remainder of the study (Woods et al., 1993). This class of antidepressants has also demonstrated efficacy in ameliorating the symptoms associated with obsessive compulsive disorder in humans (Goodman et al., 1990; Insel et al., 1990; Rapoport, 1991). Other antidepressants without effects on serotonin reuptake, such as desipramine, and antidepressants with weaker effects on serotonin reuptake such as imipramine have been demonstrated to be less effective or essentially ineffective in the treatment of obsessive compulsive disorder (Rapoport, 1991) as well as being ineffective in reducing schedule-induced polydipsia (Woods et al., 1993; unpublished observations).

Serotonin reuptake sites have been studied in binding experiments with a number of radioligands, most commonly [^{3}H]imipramine (Langer et al., 1980) and more recently [^{3}H]paroxetine (Mellerup et al., 1983). Paroxetine is one of the most potent and selective serotonin reuptake inhibitors known to date (Habert et al., 1985; Mellerup and Plenge, 1986) and labels the substrate recognition site (Marcusson et al., 1988) of the serotonin reuptake carrier.

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Since we believe schedule-induced polydipsia to be a suitable model for obsessive compulsive disorder, a biochemical analysis of brains from these animals should be useful in understanding the changes accompanying the development of schedule-induced polydipsia, and possibly in obsessive compulsive disorder. To this end, studies were undertaken to determine whether there was any evidence of changes in the serotonin reuptake carrier. Using [^{3}H]paroxetine, we looked for any changes in affinity or number of sites, in the cortex of polydipsic, non-polydipsic and fluoxetine-treated animals.

2. Materials and methods

2.1. Schedule-induced polydipsia

Polydipsia was induced in food-deprived rats (80% body weight) by exposure to a fixed-time feeding schedule (FT = 60 s; Woods et al., 1993) for 150 min daily over 3 weeks (training period). Rats were considered polydipsic when they consumed ≥ 60 ml water during the 150 min training session, whereas rats not exposed to training drank on average 17 ml during the same time period. Once trained, rats were exposed to the 150 min test session once a week. The drug treatment group received chronic administration of fluoxetine (5 mg/kg daily i.p.) for the duration of the trial (22 days). On test days, fluoxetine or the vehicle was given 60 min prior to testing. Rats representing the various treatments were killed approximately one week after the last test session. The cortex was dissected from each rat, immediately frozen on dry ice and stored at $-80°$ C until used in the [^{3}H]paroxetine binding assay. Each rat cortex was analyzed on a separate day; the length of time in the freezer was randomized among groups of animals: (i) non-polydipsic, vehicle control, food deprived; (ii) non-polydipsic, vehicle control, not food deprived (these were simply age-matched rats that had not been subjected to any training); (iii) polydipsic, vehicle control (dH_2O with 0.1 ml Tween 80); (iv) polydipsic, fluoxetine treated (5 mg/kg/day i.p. over the 22-day testing period).

2.2. [^{3}H]Paroxetine binding

The procedure was adapted from Marcusson et al. (1988). Briefly, the cortex (350–550 mg) from one animal was thawed and homogenized in 20 ml ice-cold buffer (50 mM Tris HCl containing 120 mM NaCl, 5 mM KCl; pH 7.4) using a Polytron homogenizer. The homogenate was centrifuged ($48\,000 \times g$, 10 min, 4° C), the pellet resuspended in fresh buffer and recentrifuged. The P2 pellet was resuspended in buffer to yield a final tissue concentration of 100–150 μg protein per assay tube (1.6 ml assay volume). [^{3}H]Paroxetine concentrations ranged from 0.006 to 0.5 nM; non-specific binding was defined by 10 μM fluoxetine. Filter blanks were run at each concentration. After incubation (90 min, 22° C), samples were diluted with 5 ml ice-cold buffer and filtered through Whatman GF/B filters (pretreated with 0.05% polyethyleneimine) using a Brandel cell harvester. Filters were washed 3 times with 5 ml ice-cold buffer and dried overnight before counting.

2.3. Materials

[^{3}H]Paroxetine (specific activity 21 Ci/mmol) was obtained from NEN, Boston, MA, USA. Fluoxetine HCl was a gift from Eli Lilly and Co., Indianapolis, IN, USA.

2.4. Data analysis

Binding data ($n = 3$–4 per group) were subjected to Scatchard analysis to determine the maximal number of binding sites (B_{max}) and affinity (equilibrium dissociation constant or K_d) by conventional linear least squares regression analysis. Saturation binding isotherms were also subjected to non-linear least squares fit using InPlot (v. 4.03; GraphPad) to estimate K_d and B_{max}. The Hill coefficient and Hill-derived K_d were calculated according to a method described by Bowden and Koshland (1975).

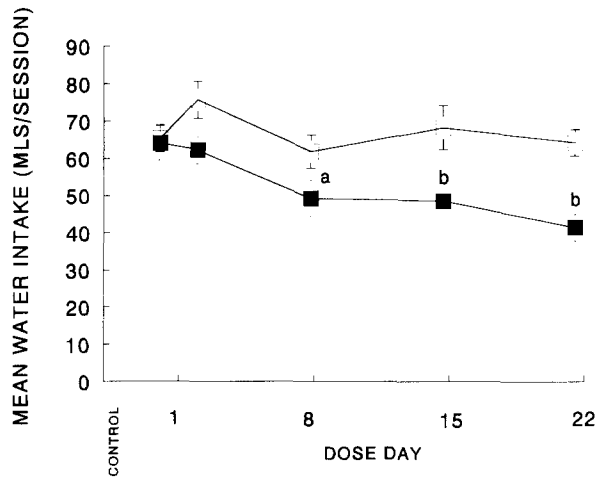

Fig. 1. The effects of fluoxetine (5 mg/kg) on the mean water intake (±S.E.M.) of polydipsic rats are illustrated. Fluoxetine or vehicle was administered once daily for 22 days with a 60-min pretreatment on test days. Control data are shown for the day prior to the commencement of dosing. $n = 3$ or 4 animals per group. [a] $P < 0.05$, [b] $P < 0.01$ compared to vehicle control. (□) Vehicle; (■) Fluoxetine 5 mg/kg.

Table 1
Summary of binding analyses by Scatchard and non-linear least squares analyses

Treatment group	n	Scatchard analysis		Non-linear least squares analysis	
		K_d (nM)	B_{max} (fmol/mg protein)	K_d (nM)	B_{max} (fmol/mg protein)
Nonpolydipsic vehicle control food deprived	4	0.078 ± 0.005 [b]	701.7 ± 144.07	0.092 ± 0.010 [a]	691.0 ± 175.6
Nonpolydipsic vehicle control not food deprived	3	0.067 ± 0.005 [b]	668.9 ± 29.59 [b]	0.083 ± 0.009 [a]	649.0 ± 23.8 [b]
Polydipsic vehicle controls	3	0.113 ± 0.004	375.5 ± 30.54	0.124 ± 0.008	366.1 ± 46.9
Polydipsic fluoxetine treated	4	0.077 ± 0.008 [a]	687.7 ± 69.06 [a]	0.091 ± 0.005 [b]	662.7 ± 87.8 [a]

t-Test: significantly different from polydipsic, vehicle control; [a] $P < 0.05$; [b] $P < 0.01$.

3. Results

Rats exposed to the training paradigm displayed schedule-induced polydipsia and this increased drinking was reduced by chronic treatment (5 mg/kg/day i.p.) with fluoxetine (Fig. 1; Woods et al., 1993). The development of polydipsia was accompanied by a 40% increase in the K_d and a 50% decrease in the B_{max} for [^{3}H]paroxetine binding (Table 1). In fluoxetine-treated polydipsic rats, these changes in K_d and B_{max} were reversed. These differences were significant when analyzed by three different methods: (1) Scatchard analysis (Table 1), (2) non-linear least squares analysis (Table 1), and (3) Hill analysis (Table 2).

4. Discussion

Detection of multiple receptor subtypes classically requires a 10-fold shift in the affinity of the radioligand for each receptor subtype (see Bennett and Yamamura, 1985). We observed a smaller but statistically significant difference in both K_d and B_{max} between polydipsic and non-polydipsic animals (with two different control groups; Table 1). In our experiments, K_d was increased by approximately 40% in polydipsic rats and B_{max} was decreased by approximately 50%. Moreover, the K_d and B_{max} values in fluoxetine-treated polydipsic animals returned to control (non-polydipsic) levels paralleling the significant reduction in polydipsic behavior (Fig. 1; Woods et al., 1993). These differences in K_d remained significant even when three methods of analysis were applied.

The Hill coefficient for binding remained close to unity (Table 2) indicating a single binding site, in agreement with Habert et al. (1985) and Marcusson et al. (1988). We expected that a shift in K_d might be indicative of the presence of two binding sites (the 'normal' serotonin reuptake carrier and a new or modified carrier induced by animals showing schedule-induced polydipsia) and that this might be reflected in a Hill coefficient of less than unity. However, it is possible that such a small change in K_d might not result in a reduction in the Hill coefficient.

We have considered several factors which may have influenced our results. The details of our assay procedures are well in line with published methods. (1) The length of time brain samples spent in the freezer was randomized. (2) It is conceivable that schedule-induced polydipsia induces an elevation of brain serotonin levels which could produce an apparent elevation in K_d; however, HPLC analysis has revealed that serotonin levels were not elevated in various brain regions of schedule-induced polydipsia animals (C.P. Smith, unpublished observations). (3) Changes in B_{max} values can affect K_d; however, B_{max} was decreased in animals exhibiting schedule-induced polydipsia and this would tend to cause an underestimation of K_d (we saw an increase). (4) Fluoxetine is known to be long acting and may have been present in the brain samples; however, the presence of fluoxetine would be expected to further *increase* K_d values (we observed a decrease to non-polydipsic control values). Thus we feel confident that the changes we observe, albeit small, are real.

Our results indicate that there is some subtle change occurring in the reuptake carrier when animals develop

Table 2
Summary of Hill analysis

Treatment group	n	Hill-derived K_d (nM)	Hill coefficient	Mean protein concentration (μg)
Nonpolydipsic vehicle control food deprived	4	0.083 ± 0.005 [b]	1.025 ± 0.009	0.094 ± 0.014
Nonpolydipsic vehicle control not food deprived	3	0.069 ± 0.006 [b]	1.020 ± 0.020	0.094 ± 0.006
Polydipsic vehicle control	3	0.116 ± 0.006	0.997 ± 0.009	0.162 ± 0.018
Polydipsic fluoxetine treated	4	0.079 ± 0.007 [b]	0.948 ± 0.073	0.106 ± 0.010

t-Test: significantly different from polydipsic, vehicle control; [a] $P < 0.05$; [b] $P < 0.01$.

polydipsia which is reversed when animals are treated chronically with fluoxetine. It is important to emphasize that these changes are the result of development of polydipsia and that food deprivation alone had no effect on either K_d or B_{max} (Tables 1 and 2). Chronic administration of serotonin reuptake inhibitors to non-polydipsic animals has been reported to have no effect on [^{3}H]paroxetine binding (Graham et al., 1987) or to increase the B_{max} (Hrdina and Vu, 1993). In addition, Chaput and his colleagues have observed an increase in the effectiveness of synaptic transmission at serotonergic synapses following chronic antidepressant treatment (see for example, Welner et al., 1989; Chaput et al., 1991); they have attributed this increase to changes in the somatodendritic and terminal serotonin autoreceptors and have measured a decrease in serotonin $(5\text{-HT})_{1A}$ receptor binding (Welner et al., 1989). The molecular basis for such changes at the synapse (for example, phosphorylation of the reuptake carrier; Blakely et al., 1991) remains obscure. It would be of interest to determine whether the actual function of the transporter (serotonin reuptake kinetics) in schedule-induced polydipsia rats is changed. Futhermore, whether such changes are reflective of differences that might be observed in patients suffering from obsessive compulsive disorder remains to be seen. Increases in glucose utilization in cortical regions in patients suffering from obsessive compulsive disorder have been reported (Baxter et al., 1992) and these changes are reversed by drugs which decrease obsessional symptoms such as fluoxetine.

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