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Extreme obesity in female rats following prepuberal induction of lithium–pilocarpine seizures and a single injection of acepromazine

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Abstract

Seizures were induced in female Wistar albino rats at either 35 or 55 days of age with a single systemic injection of lithium (3 mEq/kg) and pilocarpine (30 mg/kg); the rats were then treated with the atypical neuroleptic acepromazine (25 mg/kg). These rats manifested progressive weight gain for the rest of their lives. The effect was conspicuous by casual observation 6 weeks after treatment and occurred primarily in those rats that later developed spontaneous seizures. After 1 year, the rats were obese (>1000 g). Such weight gains, associated with almost three times the serum triglyceride levels, were not observed in male rats and have not been observed in hundreds of female rats that received this treatment as adults. Single postseizure injections of ketamine rather than acepromazine did not produce this obesity; the weights of these rats were similar to those of normal littermates. These results indicate that a *single* injection of a neuroleptic during limbic seizures before puberty can produce neuronal alterations that contribute to a lifetime of obesity.

Keywords: Obesity; Rat; Acepromazine; Seizures; Ketamine; Prepuberty

1. Introduction

In rats, hyperphagia and obesity can be induced by electrolytic lesions within the ventromedial hypothalamus or within the posterordorsal part of the medial amygdaloid nucleus [1]. The weight gain is more obvious in female rats than in male rats and has been attributed in part to the sex differences in the circulating efficacy of leptin [2,3]. Recently, Loscher et al. [4] found that twice-daily electrical kindling of the basolateral nucleus of the amygdala for up to 280 stimulations was associated with average weight gains of about 100 g compared with a group that received conventional kindling (20 stimulations) or with controls. The effect emerged about 3 weeks after the initiation of treatment and approached asymptote at about 30 weeks.

Another method [5] for inducing seizures involves a single systemic injection of either lithium (3 mEq/kg) and pilocarpine (30 mg/kg) or a larger dosage [6] of pilocarpine (about 400 mg/kg). The resultant status epilepticus produces significant mortality in adult rats (>70 days of age) unless they are injected with ketamine followed by benzodiazepines and barbiturates [7,8]. The age dependence of both survival following single lithium/pilocarpine injections and the distribution of neuropathology has been shown by Druga et al. [9]. Repeated low-dose treatment of rats with pilocarpine results in low mortality but high proportions of rats developing epilepsy [10].

In both male and female rats, the induction of epilepsy by this method leads to emergence of overt spontaneous seizures (characterized by rearing, rapid forelimb clonus, and falling) after a quiet period of between 10 and 50 days [11]. During the silent phase progressive metabolic changes underlying chronic reorganization of brain

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circuits occur in both the immature (21-day) and mature rat [12]. Behavioral sampling of these daily seizures suggests they occur at least once per day [13]. These overt behaviors are associated with long-lasting alterations in calcium homeostatic mechanisms that persist up to a year after the induction of epileptogenesis [14].

Because we have focused on the long-term behavioral consequences following the induction of epileptic seizures and the different patterns of neuronal damage produced by various postseizure treatments, we [15] have employed a variety of atypical compounds that promote survival and produce specific patterns of damage within the "neuromatrix." One of these drugs was the atypical neuroleptic acepromazine. For example, we found that maternal behavior (several months later) in rats could be abolished if acepromazine was given after induction of lithium/pilocarpine seizures [16]. A single postseizure injection of ketamine (100 mg/kg) produced comparable overall neuronal damage, but the pattern of damage within specific subcortical telencephalic and diencephalic nuclei was different from that in brains of rats that received the acepromazine. The ketamine-injected seized rats exhibited normal maternal behaviors.

While examining the potential age range in which seizures could be induced and maternal behavior would not be abolished, the first author observed the progressive adult weight gains in rats seized during prepubertal development. Casual observation showed the more obvious weight gains when seizures were induced around 35 and 55 days of age. The present experiments were designed to explore this phenomenon quantitatively. We selected offspring from both culled and unculled litters to discern if this type of earlier experience might obviously attenuate the weight gain.

2. Method

2.1. General procedure

There were five experiments over a 2-year period. They involved 127 female rats and 52 male rats. The Wistar albino breeders (the parents) were obtained at 60 days of age from Charles River. After habituating to standard wire cages in temperature (20 °C)-controlled rooms with a 12-hour light:dark cycle, the rats were bred. About 5 days before delivery, the mothers were housed in standard plastic cages with corncob bedding. Litters were weaned at 21 days of age. In all experiments Purina rat chow was available ad libitum. All analyses involved SPSS software on a VAX 4000 computer. The numbers of rats selected to be studied per experiment were calculated by the expected effect size (omega-squared estimate) of the treatment and according to the guidelines for minimal usage of animals from the Canadian Council for Animal Care.

2.2. Comparison of weights following injections at either 35 or 55 days of age

One female rat from each of 12 unculled litters (13–16 pups per litter) was injected subcutaneously with lithium chloride (3 mEq/kg, 3 mEq/cc) and, 4 hours later, with 30 mg/kg pilocarpine (30 mg/cc) in water on either Postnatal Day 35 ($n = 12$) or 55 ($n = 12$). Immediately after onset of the easily identifiable overt seizures (rearing, rapid forelimb clonus, and falling), the rats were injected subcutaneously with 25 mg/kg acepromazine (Atravert, Ayerst Labs, Montreal, Canada). Another two female rats from each of the 12 litters were injected with either only lithium ($n = 12$) or only acepromazine ($n = 12$); 6 of the rats were given the lithium only or acepromazine only on Postnatal Day 35; the others were given the treatment on Postnatal Day 55. Another 11 rats (one litter did not have any remaining females), 6 on Postnatal Day 35 and 5 on Postnatal Day 55, were simply handled (total $n = 59$). They remained in group housing (3 or 4/cage) until they were weighed at 100 days of age. Because there were no statistically significant differences between the various reference rats (lithium- or acepromazine-injected only, or handled controls), these groups were combined and defined as the control group. Two-way analysis of variance for the body weights as a function of when the rats were seized (35 vs 55 days of age) and treatment (seized plus acepromazine vs control) was completed.

2.3. Comparison of acepromazine and chlorpromazine

Two female rats from each of eight litters ($n = 16$) that had been culled to eight pups at 21 days of age were injected subcutaneously at 35 days of age with the lithium/pilocarpine solutions. Immediately after seizure onset, the rats were injected with either acepromazine (25 mg/kg, $n = 8$) or chlorpromazine (CPZ, 10 mg/kg, $n = 8$). CPZ was selected as a drug for comparison because it affects both D1 and D2 (dopamine) receptors, and this system has been implicated for the development of pseudocyesis and adiposity [17].

In a parallel study, eight rats (one from each of the same litters) were injected with lithium/pilocarpine but did not display the overt seizures, a phenomenon that occurs more frequently (for unclear reasons) in female rats. Only two rats survived and were considered a reference group to discern if they differed conspicuously from normal rats. Another eight rats (one from each litter) served as normal controls (total $n = 26$). The rats were housed singly in plastic cages containing corncob bedding and were weighed every second day after the seizure induction for 4 weeks and again after 28 weeks. One two-way analysis of variance with one within-subject level (first 4 weeks) and one between-subject level (treatments) and a single one-way analysis of variance for body weights taken 28 weeks later were completed.