ELSEVIER

Pharmacology, Biochemistry and Behavior 70 (2001) 341–352

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Synergistic induction of severe hypothermia (poikilothermia) by limbic seizures, acepromazine and physical restraint Role of noradrenergic α-1 receptors

M.A. Persinger*, R.P. O'Connor, Y.R.J. Bureau, G.H. Parker, O. Peredery, M. Zegil

Behavioral Neuroscience Laboratory, Laurentian University, Sudbury, Ontario, Canada P3E 2C6

Received 19 December 2000; received in revised form 3 May 2001; accepted 7 July 2001

Abstract

The maintained production of extreme reductions in core temperature (20–22 °C) or poikilothermia can be reliably produced by the synergistic interaction of limbic seizures (induced by lithium and pilocarpine), postseizure administration of a single injection of acepromazine, and physical restraint. Administration of the specific and nonspecific dopamine antagonists haloperidol, chlorpromazine, SCH23390, or clozapine did not simulate the effect at clinically effective dosages. Single injections of phentolamine and prazosin but not of propranolol instead of acepromazine following the seizures produced the poikilothermia. This effect was also reproduced by reducing the amount of the rats' adipose weight before the induction of the seizures and physical restraint. Rats that had been restrained or not restrained and displayed either euthermia or hypothermia exhibited significantly different patterns in brain damage within limbic and thalamic structures.

Keywords: Acepromazine; Dopamine antagonists; Limbic seizures; Adrenergic antagonists; Food deprivation; Core temperature; Brain damage

1. Introduction

About 10 years ago, while attempting to reduce the mortality of rats (Harrigan et al., 1994) in which limbic status epilepticus (Honchar et al., 1983) had been induced by a single systemic (subcutaneous) injection of lithium (3 mEq/kg) followed 4 h later by a systemic (subcutaneous) injection of pilocarpine (30 mg/kg), we (Bureau et al., 1996) found that the combination of three treatments virtually eliminated homoiothermic control. The rats that had been seized by the lithium and pilocarpine then treated with 25 mg/kg of acepromazine (Atravet) and physically restrained for 19 h exhibited marked poikilothermia.

Initially, we had restrained the rats for this period in order to help minimize the potential injury to the animals during the more severe episodes of myoclonus and to maintain the position of the sensors for electrocardiographic (ECG) measurements. From these measurements, we had planned to discern the patterns of aperiodicity and arrhythmia that often preceded the sudden cessation in cardiac activity and sudden death during the first 24 h in order to develop effective pharmacological treatments (Harrigan et al., 1994). The gradual evolution of these cardiac anomalies over several hours had been remarkably similar to the effects of delivery of electrical feedback from the ECG into the insula (Oppenheimer et al., 1991).

While monitoring cardiac activity for the first 24 h, we also noted that the overt displays of myoclonus and micromovements displayed by these rats diminished completely. The core temperatures of the rats slowly dropped to ambient (20–22 °C) levels and had approached an asymptote by about 19 h. Unless examined closely, the rats appeared to be dead. The strength of this marked hypothermic response, which was evoked *only* by the combination of seizure, restraint, and acepromazine, was equivalent to a correlation coefficient (r) of .92 (Bureau et al., 1994).

The presence of this potential pharmacological synergism and the magnitude of the effect suggested that we may have interrupted a primary pathway by which homeothermia is maintained within mammals. This possibility was particularly attractive because one reason this work had

* Corresponding author. Tel.: +1-705-675-4824; fax: +1-705-671-3844.

E-mail address: f1200002@nickel.laurentian.ca (M.A. Persinger).

0091-3057/01/$ – see front matter
PII: S0091-3057(01)00619-0

been pursued was to verify the hypothesis of MacLean (1990) concerning the critical and differentiating development within mammals of the thalamocingulate system. Previously, Peredery et al. (1992) had shown that female rats in which these seizures had been induced maintained their fecundity but did not exhibit one of the most unique features of mammals: maternal behavior. These mothers did not engage in nest building and either totally ignored or (occasionally) ate their own pups.

Within a few days of the induction of the seizures, necrosis occurs within specific clusters of neurons within about 100 structures distributed throughout the telencephalon and diencephalon (Peredery et al., 2000; Persinger et al., 1993, 1995). We had been attempting to isolate procedures by which we could experimentally remove successive stages of evolutionary progression in the organization of brain structure and thus allow expression of recondite patterns that may have existed within the premammalian or reptilian brain. The occurrence of the poikilothermia or maintained "cold blooded" response due to processes that were followed by this multifocal brain damage suggested that this model might be revealing.

The following experiments were designed to begin to identify the neurotransmitters that might mediate the extreme hypothermia or poikilothermia that was evoked by the triad of lithium/pilocarpine-induced limbic seizures, the phenothiazine acepromazine, and physical restraint (Bureau and Persinger, 1993). Our primary hypothesis was that the receptor system that mediates the electrical seizures might also be responsible for this extreme hypothermia. Considering the symptomatic similarity of changes in rats that have received agents known to be agonists of the dopaminergic D1 receptor (Barone et al., 1991) and the consequences of the lithium/pilocarpine-induced seizures, drugs were selected to pursue this mechanism. The drugs were chlorpromazine (a D1 and D2 antagonist), haloperidol (primarily a D2 antagonist), clozapine (an atypical neuroleptic that is an antagonist for dopaminergic D1 and D4 receptors as well as some subtypes of serotonergic and noradrenergic receptors; Fitton and Heel, 1990), SCH23390 (a specific D1 antagonist), and reserpine (a potent disrupter of amine-containing, presynaptic vesicles; Cooper et al., 1991).

However, on the bases of these results and data collected by other researchers (Ye et al., 1996), a pattern emerged that suggested the role of α-1 adrenergic receptors in the mediation of the poikilothermia from this powerful synergism. Consequently, we administered single injections of smaller but clinically effective dosages of compounds that are specific antagonists for the α-1 adrenergic receptor (prazosin) and for the α-2 receptor (yohimbine). Single treatments with antagonists for α-1 and α-2 adrenergic receptors (phentolamine) and β-1 and β-2 adrenergic receptors (propranolol) were employed for comparison. If the α-1 receptor subtype was the primary source of the maintained poikilothermia, we reasoned that very small dosages, compared to other antagonists, should be sufficient to evoke the poikilothermia.

2. Method

2.1. Subjects

Male ($N=365$) albino Wistar rats, between 80 and 120 days of age, were employed as subjects. They had been obtained from Charles River Breeders (Quebec) and had been habituated to a 12:12 L/D cycle (onset 07:30 h) within temperature-controlled rooms (20–22 °C) for at least 10 days.

2.2. Procedure

The general procedure was maintained for all experiments unless specified otherwise. Rats were first injected subcutaneously with 3 mEq/kg of lithium chloride (3 mEq/ml) into the hind flank. The lithium was always injected during the midlight phase, between 11:00 and 12:00 h. Four hours later, they were injected subcutaneously with 30 mg/kg of pilocarpine (Bureau et al., 1994; Persinger et al., 1993). Immediately after that injection, the rat's thoracic region was wrapped twice with 5 cm (wide) of masking tape. This procedure had been employed initially to minimize the rat's intermittent display of convulsions or severe myoclonus and to maintain the sensors for the ECG. The rat was then placed in a standard physical restrainer so that mobility was minimum (Bureau and Persinger, 1993; Harrigan et al., 1994).

The onset of the seizure, which was defined by forelimb clonus and continuous, hypertonic vibrations of the tail, was recorded to the nearest second. The display was conspicuous even when the rats were restrained in this manner. Sixty minutes after the injection of the pilocarpine, the additional pharmacological agents were subcutaneously injected into the rear flank without removing the animal from the restrainer. All doses were administered at volumes equal to 1 ml/kg.

During the subsequent 19 h, the rats were maintained within the restraint cage (between 2 and 12 rats were restrained per session) before return to the home cage. This period was selected because it was congruent with the procedures of our previous studies. Shorter durations of this particular mode of physical restraint (less than 6 h before being returned to home cage) had been associated with greater mortality. By about 18–19 h after the induction of the seizures, the rats are relatively stable. In addition, rats receiving this particular combination of lithium, pilocarpine, and acepromazine typically do not begin drinking or eating again for at least 24 h after the inductions of the seizures.

In the present study, mortality during the 19 h of restraint for any given dosage in any given experiment ranged between 0 and 2 rats and, with the exception of the group

injected with yohimbine, did not display any conspicuous treatment bias. These rats were not included in this study but constituted an additional 48 rats whose numbers were *not* included in the subject's section. For the various experiments described below, the numbers were 21 (Experiment 1), 9 (Experiment 2), 8 (Experiment 3), 10 (Experiment 4), 0 (Experiment 5; except for the yohimbine group that showed 100% mortality), 0 (Experiment 6), and 0 (Experiment 7).

Lighting from the overhead fluorescent lamps remained constant and ranged between 600 and 650 lx within the restraint area. The background sound level was constant (52 db) while the ambient magnetic field from 60 Hz power frequencies ranged between 0.1 and 0.3 mG (10–30 nT). Ambient room temperature was regulated automatically but ranged between 18 and 21 °C. Core temperature was recorded the following morning (11:00 h, after 19 h of restraint). A K-type thermocouple (resolution to 0.1 C) was inserted 10 cm into the rectum. Temperature was read from an Omega model HH21 Handheld Microprocessor Digital thermometer.

Because the ambient temperature ranged over 4 °C, we subtracted the temperature of the room (ambient) from the core temperature of the rat for our dependent measure. Although we could have employed only core temperature as the primary measure, we selected the difference score to remove the contribution from the ambient range. Analyses of variance and covariance were completed as a function of the various treatments for each experiment. Post hoc analyses employed Tukey's set at $P < .05$. All analysis involved SPSS software on a VAX 4000 computer. Except for clozapine (Sandoz) and acepromazine (Ayerst), all compounds were obtained from Sigma.

2.2.1. Experiment 1: acepromazine dose-dependence

Although 25 mg/kg of acepromazine had been employed in previous studies (Bureau et al., 1994, 1996; Bureau and Persinger, 1991, 1992, 1993; Harrigan et al., 1994; Peredery et al., 1992, 2000; Persinger, 1995a, b; Persinger et al., 1993, 1995; Persinger and Chellew-Belanger, 2001) because the rats were more likely to survive the induction of status epilepticus by the lithium and pilocarpine, the effective dosage for the synergistic production of hypothermia was not clear. This experiment was conducted to discern the dose dependence of the hypothermia contributed by this drug. After the onset of the overt seizures, rats were injected with one of the following dosages of acepromazine: 1, 2, 5, 10, 15, 25, 30, and 35 mg/kg or with isotonic saline. There were eight rats per treatment ($n = 72$).

2.2.2. Experiment 2: chlorpromazine and haloperidol

Acepromazine had initially been marketed as a major tranquilizer for human subjects shortly after the original neuroleptic, chlorpromazine, had demonstrated its potent antipsychotic effects. The potential role of D1 and D2 receptors in the production of the synergistic poikilothermia was tested by administering chlorpromazine and haloperidol. Chlorpromazine antagonizes D1 and D2 receptors while haloperidol is more specific to D2 receptors. After the onset of the seizures, rats were injected with either 10, 20, 30, or 150 mg/kg of chlorpromazine or with 2, 4, 6, or 30 mg/kg of haloperidol. There were six rats per treatment ($n = 48$).

2.2.3. Experiment 3: clozapine and SCH23390

After the onset of the overt seizures, rats were injected with either 4, 8, 12, or 120 mg/kg of clozapine. There were six rats per group ($n = 24$). Additional rats were injected with either distilled water or either 0.01, 0.3, or 1.5 mg/kg of SCH23390 (in distilled water). There were six rats per group ($n = 24$).

2.2.4. Experiment 4: reserpine

Groups of rats were injected twice per day for 3 or 2 consecutive days or the day of the injection with the lithium/pilocarpine combination with either 0.1, 0.5, 1.0, 5.0, or 10 mg/kg of reserpine. The reserpine was injected at 10:00 and 16:00 h. The sixth, fourth, and second injection for the groups injected for 3, 2, or 1 day, respectively, occurred a few minutes before the injection of the pilocarpine. There were 15 groups (3 days by five dosages) with five rats per group ($n = 75$).

2.2.5. Experiment 5: adrenergic antagonism

Thirty rats were injected with one of four (six rats per treatment) drugs: phentolamine (10 mg/kg), propranolol (10 mg/kg), prazosin (5 mg/kg) or yohimbine (5 mg/kg), or with isotonic saline. Propranolol, a β-1 and β-2 adrenergic antagonist, was included as comparison. The dosage for yohimbine that produced the high mortality was selected from the results of previous experiments by Carlisle and Stock (1995).

2.2.6. Experiment 6: prazosin dose-dependence

On the basis of the results of Experiment 5, 25 rats (five rats per dosage) were injected with either 0.05, 0.1, 1, 5, or 10 mg/kg of prazosin. If the severe hypothermia (Simmons et al., 1997) evoked by the combination of acepromazine, seizure, and physical restraint involved antagonism of the α-1 adrenergic receptor as the results of Experiment 5 implied, then this effect should be strongly dose dependent and should be evoked with relatively small concentrations.

2.2.7. Experiment 7: reduction of adipose

In light of (1) the marked simulation of the poikilothermic effect when very small amounts of the α-1 adrenergic receptor antagonist prazosin were substituted for acepromazine and (2) the known involvement of α-receptors in the activation of adipocytes (Ye et al., 1996), we hypothesized that the marked hypothermia invoked by the synergism of the seizure, physical restraint, and various drugs that shared α-1 antagonism may have been mediated through the adipocytes. In this model, the marked poikilo-

thermia may have occurred because lipolysis initiated by the combination of restraint and seizing was blocked. If this assumption was valid, then rats with minimal adipose reserves that were seized and restrained should have demonstrated comparable severe hypothermia.

Our previous measures of blood chemistry and postmortem examination of tissues and their wet weights had shown that young male rats reduced to 80% of their free-feeding weight exhibited no observable adipose reserves (Persinger et al., 1978). Most of the values for their serum electrolytes, cholesterol, and routine enzymes did not differ significantly from rats maintained on free-feed schedules. However, the food-deprived rats exhibited about one-quarter (55 mg/dl) of the level of triglycerides relative to free-feed controls (196 mg/dl).

In the present study, six rats were reduced slowly to 80% body weight over a 1 week period. Another 12 rats served as the ad libitum reference group ($n = 18$). After injection with the lithium and pilocarpine, six of the rats in the latter group were restrained and then injected with acepromazine while the other six were restrained but not injected. The rats whose body weights were 80% of their free-feed weight were injected with the lithium and pilocarpine and restrained but *not* given the acepromazine.

2.2.8. Experiment 8

Hypothermia subsequent to brain injury induced by a percussive pulse of fluid has been argued to protect the animal from sensorimotor and cognitive behavior deficits (Bramlett et al., 1995). However, blockade of the α-1 adrenoreceptor by prazosin (3 mg/kg) has also been reported to increase the behavioral deficits subsequent to traumatic brain injury induced by mechanical intrusion. Dunn-Meynell et al. (1997) suggested that this blockade during the immediate posttraumatic period encouraged excitatory transmission. It presumably exacerbated neuronal damage within pathways that were revealed by the behavioral measurements.

To discern if hypothermia was neuroprotective, as inferred by the histological integrity of the brain, weeks after the insult, we evaluated the brains of rats that had displayed the severe hypothermia and compared them to rats that had not displayed the hypothermia. We also compared rats that had been exposed to the 19 h of restraint, regardless of their thermic response, to rats that had been returned to their home cages. We reasoned this comparison might reveal the potential effects of the protracted restraint upon specific brain structures.

The brains of 40 rats from the previous experiments (Bureau and Persinger, 1992, 1993; Harrigan et al., 1994; Persinger et al., 1993, 1995) that had been injected with 3 mEq/kg of lithium chloride, 30 mg/kg of pilocarpine 4 h later, and 25 mg/kg of acepromazine 60 min later were selected from our histology library. Twenty of the rats had been restrained physically for 19 h, while the other 20 rats had not been restrained. The rats had been killed by decapitation about 2 months after the induction of the seizures. Their brains had been removed within 4 min and fixed in ethanol–formalin–acetic acid (Persinger et al., 1995).

Ten 10-μm slides equally spaced between the posterior commissure and anterior commissure were stained with toluidine blue O. The presence or absence of damage, defined as visible neuronal loss, was evaluated for each of the structures (Paxinos and Watson, 1986) within the rostral–caudal boundary of these two commissures. The evaluations were completed by experienced histopathologists who did not know the experimental conditions of each rat. Each structure was evaluated for the presence of neuronal dropout as referenced to age-matched normal (control) rats.

The proportion of damage for each structure was calculated by dividing the number of sections damaged per structure by the total number of sections evaluated for each structure (ranging from 2 to 8 depending upon structure). The validity of this measure has been reiterated by the strong (r's > .80) correlations between the amount of damage within specific structures and quantitative behavioral measures for a variety of tasks (Persinger and Chellew-Belanger, 2001; Persinger et al., 1993, 1995; Santi et al., 2001). These methods have verified the more well-known correlates between specific brain structures and specific behaviors and have revealed the potential contributions from other less well-known structures.

For analysis, the rats were designated as either restrained or not restrained. Because the hypothermia exhibited by the combination of limbic seizures, restraint, and acepromazine is usually all-or-none (severe hypothermia or poikilothermia), the rats in the restrained group were divided as well into two groups: hypothermia (core temperature between 20 and 25 °C) and euthermia or normothermia (35–38 °C). Of the 20 rats that were restrained, 12 displayed this hypothermia while 8 did not. One-way analyses of variance and nonparametric analyses (Kruskal–Wallis) as a function

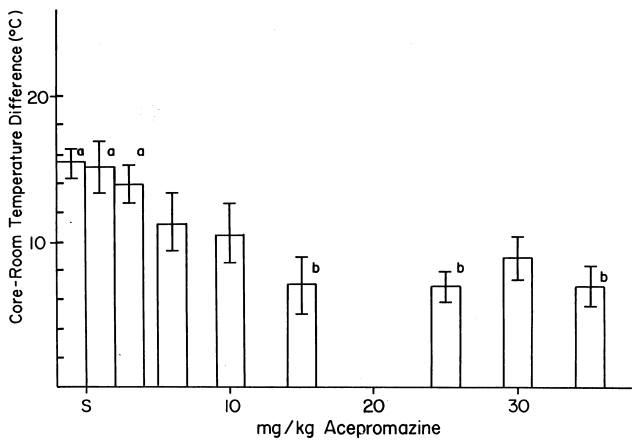

Fig. 1. Means and S.E.M. for the differences (°C) between core body temperature and room temperature as a function of dosage (mg/kg) of acepromazine (a vs. b indicates $P < .05$ significance). S = saline.

of the three groups (no restraint, restraint with no hypothermia, restraint with hypothermia) were performed for each of the approximately 100 structures within this caudal–rostral boundary that are usually affected by the seizures. To minimize inclusion of differences due to spurious effects or from outliers, only those structures that exhibited statistically ($P<.01$) differences for both the parametric and nonparametric analysis were included.

3. Results

3.1. Experiment 1

The means and standard errors of the mean (S.E.M.) for the discrepancy between the core body temperature and room temperature as a function of dosage of acepromazine are shown in Fig. 1. One-way analysis of variance demonstrated a statistically significant effect [$F(8,63)=5.07$, $P<.001$]. Post hoc analysis indicated that the major source of the difference was due to the significantly greater hypothermia for rats that had received 15, 25, and 35 mg/kg of acepromazine relative to those who received isotonic saline. Polynomial analysis demonstrated a significant linear term [$F(1,69)=8.54$, $P<.01$] as well as a superimposed small but statistically significant quadratic term [$F(1,69)=4.65$, $P<.05$] that was associated with an inflection at about 15 mg/kg of acepromazine.

3.2. Experiment 2

The means and S.E.M. for the discrepancy between core body temperature and room temperature for rats that received the dosages of haloperidol or chlorpromazine are shown in Fig. 2. One-way analysis of variance demonstrated no statistically significant difference among the groups that received the various dosages of haloperidol [$F(3,20)<1.00$, $P>.05$]. However, one-way analysis of variance demonstrated statistically significant [$F(3,20)=9.64$, $P<.01$] differences among the groups that received the dosages of chlorpromazine. The significant differences were due primarily to the greater hypothermia following the extremely large dosage (150 mg/kg) of this neuroleptic relative to rats that received either 10 or 20 mg/kg of this drug.

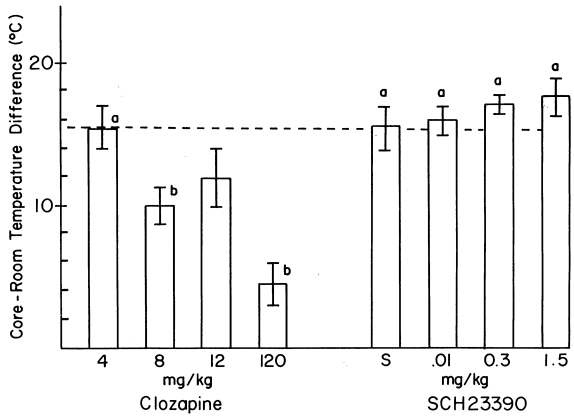

Fig. 3. Means and S.E.M. for the differences (°C) between core body temperature and room temperature as a function of dosage (mg/kg) of clozapine (clozaril) or the D1 antagonist SCH23390 (a vs. b: $P<.05$). Dotted horizontal line indicates mean value for saline controls.

3.3. Experiment 3

The means and S.E.M. for the discrepancy between core body temperature and room temperature for the primary hypothesis of this study are shown in Fig. 3. The differential

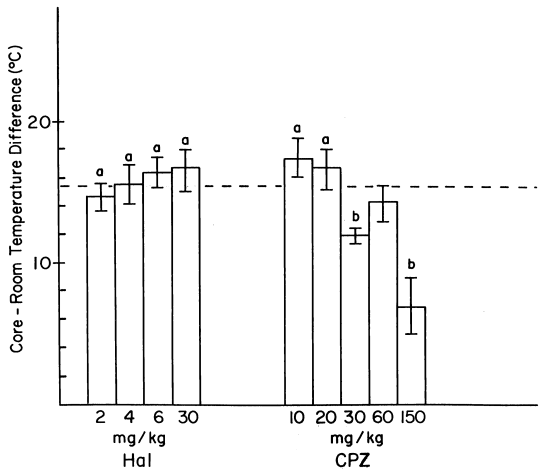

Fig. 2. Means and S.E.M. for the differences (°C) between core body temperature and room temperature as a function of dosage (mg/kg) of haloperidol (Hal) and chlorpromazine (CPZ). Dotted horizontal line indicates mean value for saline controls.

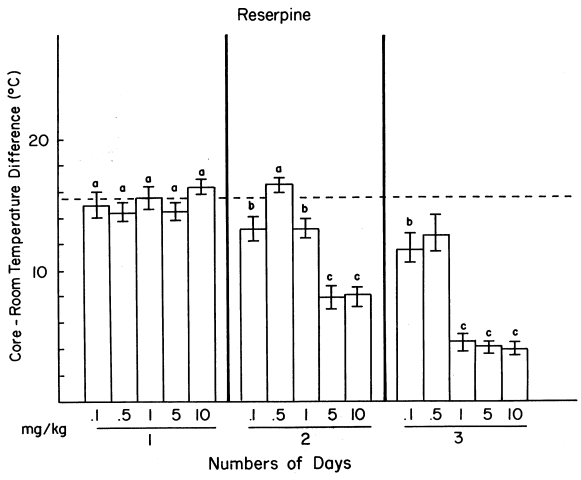

Fig. 4. Means and S.E.M. for the differences (°C) between core body temperature and room temperature as a function of numbers (days) of injection and dosage of injection (mg/kg) with reserpine. Dotted horizontal line indicates mean value for saline controls.

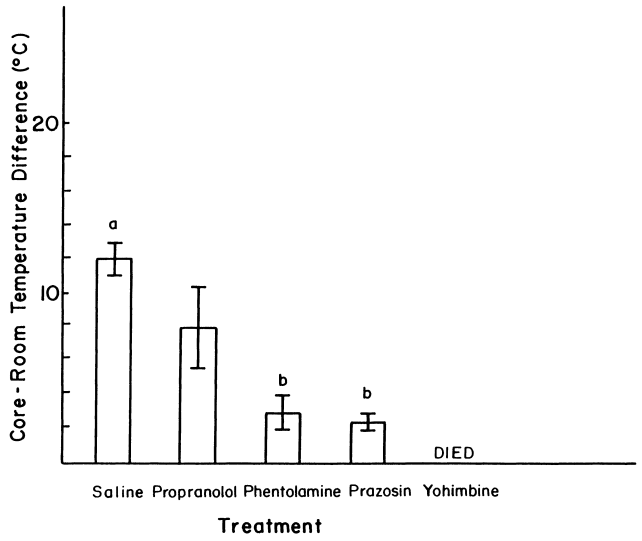

Fig. 5. Means and S.E.M. for the differences (°C) between core body temperature and room temperature associated with the α-1 adrenergic antagonist (prazosin), the α-2 antagonist (yohimbine), the α-1 and α-2 antagonist (phentolamine), the β-1 and β-2 adrenergic antagonist (propranolol), or saline.

dosages of clozapine resulted in significant differences [$F(3,23) = 10.15$, $P < .001$] which were due primarily to the greater hypothermia in rats that received the 8 and 120 mg/kg of clozapine relative to those who received 4 mg/kg of this atypical neuroleptic. On the other hand, there were no statistically significant differences among the groups that received the different dosages of the specific D1 dopaminergic antagonist SCH23390 [$F(3,26) = 0.71$, $P > .05$].

3.4. Experiment 4

Two-way analysis of variance as a function of days of injections of the reserpine and the dosages of injection demonstrated that the larger effect size for the discrepancy between core temperature and room temperature was due to the numbers of (days) injections [$F(2,60) = 33.09$, $P < .001$] before the induction of the seizures. The contribution from dosage [$F(4,60) = 7.09$, $P < .01$] and the interaction between dosage and days [$F(8,60) = 3.06$, $P < .01$] were both significant statistically. As shown in Fig. 4 and verified by post hoc analysis, the primary source of the interaction was due to the greater hypothermic effects from the 5 and 10 mg/kg dosages after 2 days of treatment and from these two dosages plus the 1 mg/kg amount after 3 days of treatment relative to the groups that received the reserpine on the same day as the induction of the seizures.

The linear combination of dosage and numbers of days of treatment explained 50% of the variance in the hypothermia (multiple $r = .71$). Covariance for body weight before the two-way analysis of variance was completed did not significantly or appreciably alter the effect sizes for dosage or days of treatment. The contribution from concurrent body weight to the hypothermia (regression coefficient, i.e., slope = 0.03) added an additional 5% to the total explained variability in the discrepancy between core temperature and room temperature.

3.5. Experiment 5

The means and S.E.M. for the differences between core and ambient temperature for rats injected with saline, propranolol, phentolamine, prazosin, or yohimbine are shown in Fig. 5. One-way analysis of variance demonstrated a statistically significant group difference [$F(3,24) = 8.62$, $P < .001$]. This difference explained 58% of the variance in this measure. Post hoc analysis indicated more poikilothermia was displayed by the phentolamine and prazosin groups relative to the groups that received propranolol or saline. All of the rats receiving this dosage of yohimbine died.

3.6. Experiment 6

The means and S.E.M. for the degree of poikilothermia, as inferred by the discrepancy between the core temperature and room temperature, as a function of dosage of prazosin are shown in Fig. 6. One-way analysis of variance indicated a statistically significant difference between the groups [$F(4,19) = 16.63$, $P < .001$]. The treatments explained 84% of the variance in the hypothermic measures. Polynomial analysis indicated that the dose dependence was significantly linear [$F = 61.55$, $P < .001$].

3.7. Experiment 7

The means and standard deviations (S.D.) for the core temperatures groups that were seized and restrained, seized, administered acepromazine, and restrained, and food deprived, seized, and restrained are shown in Table 1.

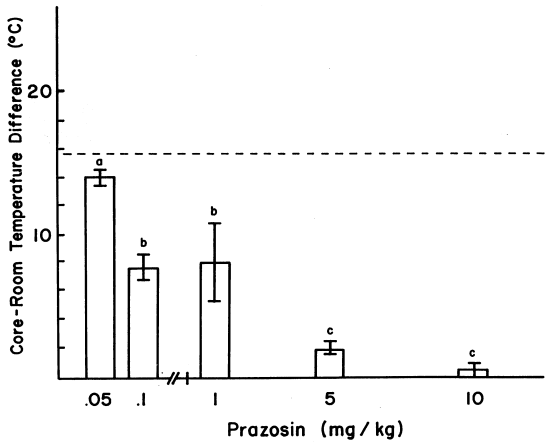

Fig. 6. Means and S.E.M. for the differences (°C) between core body temperature and room temperature in rats that were seized and restrained as a function of dosage of prazosin. Dotted horizontal line indicates mean value for saline controls.

Table 1
Means and S.D. for the core temperatures (°C) of groups of rats ($n=6$ per group) that were seized and restrained, seized, administered acepromazine, and restrained, or reduced to 80% body weight, seized, and restrained

Group	Mean core temperature (°C)	S.D.
Seized-restrained	35.9	2.6
Seized-acepromazine-restrained	23.9	1.6
Food deprived-seized-restrained	24.5	1.2

One-way analysis of variance demonstrated a statistically significant group difference [$F(2,15)=75.68$, $P<.001$] that explained 91% of the variance in the core temperatures. Post hoc analysis indicated that the core temperatures displayed by the rats that were food deprived, seized, and restrained did not differ significantly from the core temperatures of the rats that had received the usual procedure (seizure, acepromazine, and restraint). Both groups displayed significantly lower body temperatures than the group that was seized and restrained only. This group's core temperature was within the range of normal nontreated rats.

3.8. Experiment 8

The means and S.D. for the averaged proportion of sectors displaying damage for *all* structures for the brains of rats within the three groups were: no restraint: 27 (7), restraint with minimal hypothermia: 20 (8), and restraint with extreme hypothermia: 25 (7). These differences between the three groups were marginally significant statistically [$F(2,37)=3.06$, $P=.05$]. Post hoc analysis (Tukey's $P<.05$) indicated that this difference was due to the less general damage within the brains that had been restrained but did not exhibit hypothermia compared to those that were not restrained. The qualitative characteristics of the neuronal and glial changes were similar to those reported in previous studies (Peredery et al., 2000; Persinger et al., 1993) and were restricted to the diencephalon, limbic telencephalon, and neocortices.

The means and S.D. for the proportion of sections containing neuronal loss for the most statistically significant ($P<.01$) structures as defined by both parametric and nonparametric one-way analyses of variance are shown in Table 2. The effect size or η^2 defined as the amount of variance in the damage scores accommodated by the three treatments (not restrained, restrained without hypothermia, and restrained with hypothermia) is also shown for each structure. Although several dozens of structures within the rostral–caudal extent of the volume were damaged as usual, the differential damage occurred primarily within specific regions of the hippocampal–amygdaloid complex, the posterior thalamus, and the central thalamus.

For example, the rats that were restrained and displayed hypothermia displayed significantly more damage within the inner blade of the dentate gyrus (DG) than rats that were either restrained and did not display hypothermia or were not restrained (and were euthermic). However, most of the statistically significant differences, indicated by the superscripts, were associated with the rats that had been restrained and had *not* displayed hypothermia relative to those who were restrained and had displayed hypothermia or that had not been restrained.

Table 2
Means and S.D. for structures of the brains of rats that displayed both parametric and nonparametric significant ($P<.01$) differences in proportions of sections containing neuronal loss for rats that were not restrained, restrained and displayed no hypothermia, or restrained and displayed hypothermia (<30 °C core temperature) following status epilepticus by lithium/pilocarpine

			Restraint hypothermia				
	No restraint ($n=20$)		No ($n=8$)		Yes ($n=12$)		η^2
	M	S.D.	*M*	S.D.	*M*	S.D.	(%)
Structure							
DG	24[a]	33	17[a]	24	63[b]	29	28
CA4 field hippocampus	42[a]	34	27[a]	32	72[b]	28	21
Inner blade of DG	11[a]	32	17[a]	41	79[b]	33	44
Ventral endopiriform nuc.	96[a]	8	73[b]	14	91[a]	10	26
Basolateral amygdala nuc. (anterior part)	65[a]	30	20[b]	24	46	42	21
Basolateral amygdala nuc. (ventral part)	85[a]	29	32[b]	41	87[a]	25	30
Lateral amygdala nuc. (ventrolateral part)	86[a]	28	17[b]	40	62[a]	37	23
Dorsolateral geniculate	62[a]	31	5[b]	12	58[a]	37	25
Medial geniculate nuc. (ventral part)	35[a]	37	0[b]	0	17	31	20
Medial geniculate nuc. (medial part)	38[a]	39	0[b]	0	17	32	20
Posterior thalamic nuc. (triangular part)	56[a]	40	8[b]	20	24	38	26
Centrolateral thalamic nuc.	53[a]	35	0[b]	0	25[b]	23	23
Gelatinous thalamic nuc.	52[a]	42	8[b]	24	19[b]	38	20

η^2 indicates the amount of variance accommodated by the three conditions.

Table 3
Relevant statistics, Wilks' λ (amount of variance remaining to be explained), change in Rao's *V*, and the standardized canonical discriminant function coefficient (SCDFC) for brain structures that discriminated rats that were not restrained or restrained for 19 h following induction of status epilepticus

Variable	Wilks' λ	Change in *V*	SCDFC
Inner blade of DG	0.73	13.46	1.24
Centrolateral thalamic nuc.	0.51	22.25	−1.01
Anteroventral thalamic nuc. (dorsomedial part)	0.35	27.56	−0.81
Parietal Ctx (area 2)	0.27	33.21	−0.78
Intergeniculate leaf	0.21	35.59	−0.60

Canonical correlation = .89. 100% correct classification. $\chi^2(5)=52.42$ (valences of SCDFC indicate actual direction of partial slopes).

Table 4
Relevant statistics, Wilks' λ (amount of variance remaining to be explained), change in Rao's *V*, and the SCDFC for brain structures that discriminated restrained rats that did not display or displayed severe hypothermia following induction of status epilepticus

Variable	Wilks' λ	Change in *V*	SCDFC
DG	0.54	13.32	1.88
Lateral amygdala nuc. (dorsolateral part)	0.32	20.32	1.97
Lateroposterior thalamic nuc. (laterocaudal part)	0.14	61.44	1.72
Medial amygdaloid nuc. (anterioventral part)	0.08	78.06	0.91

Canonical correlation = .95. $\chi^2(4) = 34.51$. 100% correct classification.

To discern if these multiple structures simply reflected the same source of variance, discriminant analyses were completed between rats that were restrained or not restrained (regardless of hypothermia) and those that displayed hypothermia (regardless of restraint). Because stepwise discriminant analysis does not allow inclusion of redundant sources of variance, the variables that entered the function were considered independent. The numbers of steps were restricted to 5 because of the sample size ($n = 40$).

The variables that entered the discriminant function for rats that were not restrained or restrained are shown in Table 3. The function indicated that the rats that were restrained, compared to nonrestrained, could be discriminated with 100% accuracy by their enhanced damage in the inner blade of the dentate gyrus but less damage in centrolateral thalamus, anteroventral thalamus, area 2 of the parietal cortices, and the intergeniculate leaf.

The variables that entered the function for the rats that were euthermic or displayed the severe hypothermia also correctly classified 100% of the two groups. The rats that displayed the hypothermia also displayed *more* neuronal loss within the DG, lateral amygdaloid nucleus, lateroposterior thalamic nucleus, and medial amygdaloid nucleus. The linear combination of weighted scores for the first two structures that entered the function classified all but one of the rats (Table 4).

4. Discussion

As reported previously (Bureau and Persinger, 1993), the synergistic interaction between (1) the induction of limbic seizures by lithium concentrations within the human therapeutic range and the muscarinic agent pilocarpine, (2) physical restraint, and (3) the injection of acepromazine resulted in a poikilothermia. This condition developed within a few hours and was still present 19 h later. At that time and for at least an additional day after they were removed from the restraints and returned to their home cages or to a recuperating cage, the rats were immobile, cold to the touch, and appeared dead. The magnitude and duration of the hypothermia was more extreme than many other models of hypothermia (e.g., Buchan and Pulsinelli, 1990).

We had initially selected acepromazine as a postseizure treatment because it had been the pharmacological agent that reduced maximally the typical mortality following lithium/pilocarpine-induced seizures. [Recently, ketamine has been shown to be comparably effective and to be associated with less deficits for spatial learning and memory (Santi et al., 2001)]. When the rats were restrained, in order to help minimize the distress and potential injury from intermittent myoclonus, we then found a reduction of body temperature that approached ambient room temperatures (about 20 °C). This severe hypothermia, or more accurately poikilothermia, was maintained for up to 3 days after the application of the three treatments particularly when reserpine was employed.

The results of the present study indicated that this synergism involved α-1 adrenergic receptors. Acepromazine, a nonspecific antagonist of α-receptors (Brock, 1994), facilitated the poikilothermia when the dosages exceeded 15 mg/kg. Experiment 1 showed no appreciable change in the degree of hypothermia even at higher dosages. Simplistically, this weak linear effect would be more typical of drugs that affect more than one receptor subtype or that exhibit noncomplete saturation of the receptor subtype.

Our original hypothesis, derived from Barone et al.'s (1991) results that showed D1 dopaminergic agonists facilitated and D1 antagonists inhibited the overt display of these limbic seizures, did not support the primary role of this receptor system in this form of poikilothermia. The specific D1 antagonist SCH23390 failed to produce the effect. Chlorpromazine, an antagonist of both D1 and D2 receptors, produced poikilothermia comparable to those associated with the acepromazine. However, extraordinarily large dosages, 150 mg/kg, were required. Haloperidol, which displays a preferential antagonism for D2 receptors (Al-Tajair and Starr, 1991), was not effective with any of the dosages employed.

Similarly, the atypical neuroleptic clozapine produced the marked poikilothermia when the dosage was 120 mg/kg. The occurrence of a mild hypothermia, of about 4 °C, in response to the seizure, restraint, and 8 mg/kg of the drug suggested that this multiple-receptor influencing compound may have been mediating its effects through nondopamine receptors rather than through D4 receptors or their variants.

Although the results minimized the likelihood that D1 receptors mediated this type of hypothermia, the role of catechol-derived neurotransmitters is clear. Reserpine, injected twice per day for 3 days, when dosages were equal to or exceeded 1 mg/kg also evoked poikilothermia that was comparable to the effects of acepromazine. That the hypothermia was not evident during the first 24 h after any of the dosages of reserpine but required at least 2 days to evolve indicates that this rauwolfia derivative was not acting at the same receptor sites as the compounds employed in the first three experiments. The time lag of 2 days, before the

hypothermia emerged, is consistent with the consequences of depletion or destruction of the synaptic vesicles that contain the neurotransmitters (Cooper et al., 1991). Alternatively, a number of different catecholamine neurotransmitters may have been released simultaneously by this historical compound and may have induced a synergism with similar overt characteristics.

The simulation of the magnitude of the poikilothermia produced by higher dosages of acepromazine was produced by pharmacological agents that are antagonists of either α-1 and α-2 (phentolamine) or α-1 adrenergic receptors (prazosin) only. We cannot totally eliminate the possibility that yohimbine, an α-2 antagonist, may have produced this poikilothermia because all of the rats died. Further research must be completed to discern if the death was mediated by cardiac arrhythmias or extreme poikilothermia that interfered with the contractile properties of the heart because of the enhanced viscosity in blood flow.

An integration of the results of these experiments and our more recent research indicates that this form of hypothermia is the consequence of the acepromazine's inhibition of gluconeogensis. We hypothesize the following. As the massive neuroelectrical activity that accompanies limbic status epilepticus continues, more energy is required. Physical restraint, which is associated with a marked elevation in the release of noradrenaline, adrenocorticotropin, and corticosterone (Amar and Sanyal, 1981; Glavin et al., 1994), activates the lipogenic, metabolic pathways (Plotsky et al., 1989). Indirect evidence for this activation has been the repeated observation of loss of between 10% and 20% of the body (primarily adipose) weight by these rats during the first 48 h after the seizure induction, during which time they are relatively motionless within their home cages. Although these rats do not eat during this period, the loss exceeds that associated with normal food deprivation even for active rats.

If brain and body metabolism becomes primarily dependent upon gluconeogenesis, i.e., lipolytic sources of energy, then one would expect that rats that displayed the greatest relative reserves of adipose to demonstrate an attenuation of hypothermia. This contention was supported in the present study by the significant positive correlation between the rats that lost the least amount of weights during restraint and the more pronounced poikilothermia, i.e., maximum difference between core and room temperature. In addition, the rats whose body weights had been reduced to 80% of free-feed weight before they were injected with lithium and pilocarpine and then restrained also showed the marked hypothermia even without the injection of acepromazine or any other substance. If these observations are integrated, then the most logical conclusion is that acepromazine inhibits activity of α-1 adrenergic (or closely related) receptors on adipocytes and consequently prevents the necessary gluconeogenesis that would have prevented the hypothermia. Similar mechanisms have been hypothesized for thermogenesis of brown adipose in rodents (Borst et al., 1994).

There are other potential interpretations of our results that may involve more central mechanisms within the hypothalamus or the combined effects of hypothalamic functions and shivering (Hayashi, 1983; Shimada and Stitt, 1983; Ye et al., 1996). The results of the present study are also not strongly supportive of the use of this poikilothermic response from the synergism of seizure restraint and antagonism of α-1 receptors as a tool to reveal within the mammalian brain the recondite patterns of reptilian neuronal organization that may still remain but be suppressed by more recent evolutionary developments.

There is a practical implication of the marked hypothermia following a catastrophic neurochemical condition such as the precipitation of status epilepticus by lithium and pilocarpine, physical restraint, and the injection of α-1 antagonists (Szreder, 1992). It may be relevant to specific cases of brain injury in humans and other animals. For example, Dunn-Meynell et al. (1997) found that blockade of α-1 adrenergic receptors during the immediate post-trauma period lead to enhancement of excitatory neurotransmission, which exacerbated the subsequent behavioral deficits. Because various degrees of physical restraint of human beings within stretchers or emergency wards may follow emergency pharmacological treatment after traumatic, closed head injuries (that can be associated with limbic lability as inferred by the classic retrograde amnesia of between 10 and 20 min), the relevance of our observations to these settings should be considered.

The histopathological results suggest that the physiological (body temperature) and physical (restraint) environment subsequent to the induction of status epilepticus may not strongly affect the averaged damage within brain space. The proportion of slides for all structures that were damaged by the seizures was about 25% for all three conditions. However, these conditions clearly affected the pattern and the severity of neuronal loss within the brain. Cook and Persinger (1996) have found that very subtle stimuli, such as transcerebral exposures to weak (1 μT) complex, pulsed magnetic fields during the first 19 h after the induction of the seizures while the rats were restrained, may affect neuronal viability.

In the present study, the structures that accurately classified the rats that had been either restrained or not restrained after the induction of the seizures were not the same as those that accurately discriminated the rats that had been restrained and either displayed extreme hypothermia or minimal hypothermia. The strength of the canonical correlations, the 100% accuracy of classification, and the substantial amount of variance explained by even the first *two* variables that entered each of the two functions indicate that these two different conditions (restraint vs. nonrestraint and restraint with hypothermia vs. restraint with minimal hypothermia) affected the viability of neurons within quite different structures.

If one assumes permanent disruptions of neuronal density and integrity following a trauma is one index of cellular stress sustained during the trauma, then the protracted

physical restraint (without extreme hypothermia) for 19 h may have produced a mild protection. As noted in Table 2, the rats that were restrained and did not display extreme hypothermia showed less neuronal loss in the ventral endopiriform nucleus, basolateral amygdala (anterior and ventral part), lateral amygdala (ventrolateral part), dorsal lateral geniculate, medial geniculate (ventral and medial part), posterior thalamic nucleus, centrolateral thalamic nucleus, and gelatinous thalamic nucleus. Considering the intrinsic connections between some of these structures (McIntyre and Plant, 1989; Miller, 1992; Mirski et al., 1986) and their contribution to the spread of electrical seizures, we suspect that the maintained physical restraint during the first 19 h after the induction of the seizures may have reduced the excitotoxic effects of excessive activity within these structures.

That somatic stimuli can inhibit the spread of some seizures is not unprecedented. For example, the spread of the paroxysmal discharges along the homonculus, the "Jacksonian March," can be disrupted in some episodes by tactile and proprioceptive input evoked by strong grasping of the limb anterior to the path of the focal contractions (Dreifuss and Lee, 1981). In the present study, the function that accurately discriminated rats that were restrained for 19 h compared to those that were not indicated that the restrained rats also exhibited less neuronal dropout within area 2 of the parietal cortices (Table 3) in context of the scores for the inner blade of the DG, the centrolateral nucleus and anteroventral nucleus of the thalamus, and the intergeniculate leaflet. This area of the parietal cortices becomes activated with forelimb movement (Ebrahimi-Gaillard et al., 1994). Reduction of cell loss in this particular region suggests that restraint may have helped reduce the neuronal damage accompanying excessive forelimb clonus during the protracted seizures.

There was no simple evidence that the extreme hypothermia conspicuously and adversely affected the structures specifically within the thalamocingulate system. If one assumes the maintained loss of mammalian euthermia was associated with excitotoxic incapacitation of neurons that was later reflected as neuronal dropout, then only the DG and CA4 region of the hippocampus was involved. Only these structures exhibited significantly more neuronal loss compared to rats that were restrained and did not display the extreme hypothermia or were not restrained.

Consistent with this explanation are the measurements by Motte et al. (1998) that immunoreactivity for HSP72, the heat shock protein, only occurred about 50 min after the induction of lithium/pilocarpine seizures and began first in the polymorphic layer of the DG. If we assume that the elevations of prostaglandin F α-2 within the hippocampus (Naffah-Mazzacoratti et al., 1995) during status epilepticus are associated with their anticonvulsant effects (Naffah-Mazzacoratti et al., 1995), then the attenuation of the levels of this derivative of arachidonic acid after moderate (30 °C) hypothermia (Patel et al., 1994) might explain the greater neuronal loss observed in our study within the dentate region.

It may be relevant that the DG and CA3 regions of the rat and human brain are remarkably capable of reactive synaptogenesis. Synaptic reorganization of mossy fibers within the human temporal lobe occurs with the sprouting of mossy fiber axons into the denervated zone within the hilar neurons of CA4 and the inner molecular layer of the DG (Sutula et al., 1989; Simmons et al., 1997). The quiet period after the induction of lithium/pilocarpine seizures in rats that can range from about 4 to 44 days (Naffah-Mazzacoratti et al., 1995) is associated with intense sprouting of mossy fibers into the inner molecular layer. The concomitant increase in spontaneous excitatory currents and inhibition of κ-opiate receptors in the DG (Simmons et al., 1997) have been suggested as a major cause for the emergence of spontaneous seizures that occur intermittently for the remainder of the animal's life.

Rats that displayed the more extreme hypothermia have exhibited more frequent overt spontaneous seizures during their lifetime compared to those rats that did not (unpublished observations). These rats become more sensitive to conditioned seizures, our model of pseudoseizures, or psychogenic seizures, when sounds are paired with daily presentation of food (Persinger and Chellew-Belanger, 2001). The occurrence of the spontaneous seizures in these rats is also strongly correlated with increases in global geomagnetic activity (Michon and Persinger, 1997; Persinger, 1995b). We have hypothesized that the sensitivity of these seizures to environmental stimuli involves the suppression of melatonin and the release of corticotropin releasing factor, which can be an epileptogen, and adrenocorticotropic hormone (Persinger and Chellew-Belanger, 2001).

What is not known at this time is whether or not the areas of brain damage or protection associated with the hypothermia induced by the specific combination of lithium/pilocarpine-induced seizures, physical restraint, and acepromazine would also be the same as those evoked by the seizures, restraint, and the antagonists of α-1 receptors or the induction of seizures and physical restraint of rats in which adipose levels have been substantially reduced. Experiments designed to answer this question might reveal if the blockade of specific receptors or groups of receptors with different pharmacological agents result in permanent changes in the proportions of neurons within the same specific structures of the brain.

Acknowledgments

We thank Dr. Andre Michon for his technical assistance. The suggestions of Dr. G.B. Glavin, Department of Pharmacology and Therapeutics, University of Manitoba, and Bruce McKay were appreciated. Thanks to Sandoz Canada (Dorval, Quebec) for supplying the

clozapine and to Ayerst Laboratories (Montreal, Quebec) for supplying the acepromazine.

References

Al-Tajair G, Starr MS. D-2 agonists protect rodents against pilocarpine-induced convulsions by stimulating D-2 receptors in the striatum, but not in the substantia nigra. Pharmacology 1991;39:109–13.

Amar A, Sanyal AK. Immobilization stress in rats: on rectal temperature and possible role of brain monoamines in hypothermia. Psychopharmacology 1981;73:157–60.

Barone P, Palma V, DeBartolomeis A, Tedeschi E, Muscettola G, Campanella G. Dopamine D1 and D2 receptors mediate opposite functions in seizures induced by lithium–pilocarpine. Eur J Pharmacol 1991; 195:157–62.

Borst SE, Oliver RJ, Sego RL, Scarpace PJ. Alpha-adrenergic receptor-mediated thermogenesis in brown adipose tissue of rat. Gen Pharmacol 1994;25:1703–10.

Bramlett HM, Green EJ, Dietrich D, Busto R, Globus MY-T, Ginsberg M. Posttraumatic brain hypothermia provides protection from sensorimotor and cognitive behavioral deficits. J Neurotrauma 1995;12:289–98.

Brock N. Acepromazine revisited. Can Vet J 1994;35:458–9.

Buchan A, Pulsinelli WA. Hypothermia but not the *N*-methyl-D-aspartate antagonist, MK-801, attenuates neural damage in gerbils subjected to transient global ischemia. J Neurosci 1990;10:311–8.

Bureau YRJ, Persinger MA. Cholinergic rebound after chlorpromazine exacerbates lithium muscarinic-induced limbic seizures in rats: implications for psychiatric treatment. Psychol Rep 1991;69:171–6.

Bureau YRJ, Persinger MA. Geomagnetic activity and enhanced mortality in rats with acute (epileptic) limbic liability. Int J Biometeorol 1992; 36:226–32.

Bureau YRJ, Persinger MA. Extreme hypothermia induced by a synergism of acute limbic seizures, physical restraint and acepromazine: implications for survival following brain injury. Psychol Rep 1993;72:248–50.

Bureau YRJ, Peredery O, Persinger MA. Concordance of quantitative damage within the diencephalon and telencephalon following systemic pilocarpine (380 mg/kg) or lithium (3 mEq/kg)/pilocarpine (30 mg/kg) induced seizures. Brain Res 1994;648:265–9.

Bureau YRJ, Persinger MA, Parker GH. Effect of enhanced geomagnetic activity on hypothermia and mortality in rats. Int J Biometeorol 1996; 39:197–200.

Carlisle HJ, Stock MJ. Temperature-dependent effects of alpha adrenergic agonists and antagonists in the cold. Pharmacol Biochem Behav 1995; 51:263–70.

Cook LL, Persinger MA. Long-term consequences of subtle stimuli during the first twenty-four hours of seizure-induced brain injury. Percept Mot Skills 1996;83:523–9.

Cooper JR, Bloom FE, Roth RH. The biochemical basis of neuropharmacology. 6th ed. New York: Oxford Univ. Press, 1991.

Dreifuss FE, Lee SI. Epilepsy case studies. Garden City, NY: Medical Examination Publishing, 1981.

Dunn-Meynell AA, Yarlagadda Y, Levin BE. alpha1-adrenoreceptor blockade increases behavioral deficits in traumatic brain injury. J Neurotrauma 1997;14:43–52.

Ebrahimi-Gaillard A, Beck T, Wree A, Roger M. Metabolic mapping of the forelimb motor system in the rat: local cerebral glucose utilization following execution of forelimb movements mainly involving proximal musculature. Somatosens Mot Res 1994;11:229–41.

Fitton A, Heel RC. Clozapine: a review of its pharmacological properties and therapeutic use in schizophrenia. Drugs 1990;40:722–47.

Glavin GB, Pare WP, Sandbak T, Bakke H, Murison R. Restraint stress in bio-medical research: an update. Neurosci Biobehav Rev 1994;18: 223–49.

Harrigan T, Bureau YRJ, Persinger MA, Parker GH. Prevention of sudden cardiac death by the atypical neuroleptic acepromazine following status epilepticus in rats. Life Sci 1994;54:457–62.

Hayashi M. Restraint-induced thermogenesis blunted by fasting in rats. Am J Physiol 1983;244:323–8.

Honchar MP, Olney JW, Sherman WR. Systemic cholinergic agents induce seizures and brain damage in lithium-treated rats. Science 1983;220: 323–5.

MacLean PD. The triune brain in evolution: role in paleocerebral functions. New York: Plenum, 1990.

McIntyre DC, Plant JR. Pyriform cortex involvement in kindling. Neurosci Biobehav Rev 1989;13:277–80.

Michon AL, Persinger MA. Experimental simulation of the effects of increased geomagnetic activity upon nocturnal seizures in epileptic rats. Neurosci Lett 1997;224:53–6.

Miller JW. The role of mesencephalic and thalamic arousal systems in experimental seizures. Prog Neurobiol 1992;39:155–78.

Mirski MA, McKeon AC, Ferrendelli JA. Anterior thalamus and substantia nigra: two distinct structures mediating experimental generalized seizures. Brain Res 1986;397:377–80.

Motte J, da Silva Fernandes MJ, Baram TZ, Nehlig A. Spatial and temporal evolution of neuronal activation, stress and injury in lithium–pilocarpine seizures in adult rats. Brain Res 1998;793:61–72.

Naffah-Mazzacoratti MG, Bellissimo MI, Cavalheiro EA. Profile of prostaglandin levels in the rat hippocampus in pilocarpine model of epilepsy. Neurochem Int 1995;27:461–6.

Oppenheimer SM, Wilson JX, Guiraudon C, Cechetto DF. Insular cortex stimulation produces lethal cardiac arrhythmias: a mechanism of sudden death? Brain Res 1991;550:115–21.

Patel PM, Drummond JC, Cole DJ, Yaksh TL. Differential temperature sensitivity of ischemia-induced glutamate release and eicosanoid production in rats. Brain Res 1994;650:205–11.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 2nd ed. Orlando, FL: Academic Press, 1986.

Peredery O, Persinger MA, Blomme C, Parker G. Absence of maternal behavior in rats with lithium/pilocarpine seizure-induced brain damage: support for MacLean's triune brain theory. Physiol Behav 1992;52: 665–71.

Peredery O, Persinger MA, Parker G, Mastrosov L. Temporal changes in neuronal dropout following inductions of lithium/pilocarpine seizures in the rat. Brain Res 2000;881:9–17.

Persinger MA. Sudden unexpected death in epileptics following sudden, intense, increases in geomagnetic activity: prevalence of effect and potential mechanisms. Int J Biometeorol 1995a;38:180–7.

Persinger MA. Geophysical variables and behavior: LXXIX. Overt limbic seizures are associated with concurrent and premidscotophase geomagnetic activity: synchronization by prenocturnal feeding. Percept Mot Skills 1995b;81:83–93.

Persinger MA, Chellew-Belanger G. Synchronized feeding as a "conditioned stimulus" for overt seizures in chronically (limbic) epileptic rats: a model for "psychogenic seizures" with complex partial epilepsy. Int J Neurosci 2001;106:169–184.

Persinger MA, Carrey NJ, Lafreniere GF, Mazzuchin A. Step-like DRL schedule change effects on blood chemistry, leucocytes, and tissue in rats. Physiol Behav 1978;21:899–904.

Persinger MA, Bureau YRJ, Kostakos M, Peredery O, Falter H. Behaviors of rats with insidious, multifocal brain damage induced by seizures following single peripheral injections of lithium and pilocarpine. Physiol Behav 1993;53:849–66.

Persinger MA, Bureau YRJ, Peredery O. Dissociation between conditioned taste aversion and radial maze learning following seizure-induced multifocal brain damage: quantitative tests of serial vs. parallel circuit models of memory. Physiol Behav 1995;56:225–35.

Plotsky PM, Cunningham ET, Widmaier EP. Catecholaminergic modulation of corticotrophin releasing factor and adrenocorticotropic secretion. Endocr Rev 1989;10:437–58.

Santi SA, Cook LL, Persinger MA, O'Connor RP. Normal spatial memory following postseizure treatment with ketamine: selective damage attenu-

ates memory deficits in brain-damaged rodents. Int J Neurosci 2001; 107:63–75.

Shimada SG, Stitt JT. Inhibition of shivering during restraint hypothermia. Can J Physiol Pharmacol 1983;61:977–82.

Simmons ML, Terman GW, Chavkin C. Spontaneous excitatory currents and kappa-opiate receptor inhibition in dentate gyrus are increased in rat pilocarpine model of temporal lobe epilepsy. J Neurophysiol 1997;78: 1860–8.

Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. Ann Neurol 1989;26:321–30.

Szreder Z. Do central mechanisms participate in the thermoregulatory activity of prazosin? Gen Pharmacol 1992;23:1121–4.

Ye J-M, Edwards SJ, Rose RW, Steen JT, Clark MG, Colquhoun EQ. Alpha-adrenergic stimulation of thermogenesis in a rat kangaroo (marsupialia, *Bettongia gaimardi*). Am J Physiol 1996;40:586–92.