ELSEVIER

Pharmacology Biochemistry & Behavior, Vol. 55, No. 2, pp. 185–194, 1996

0091-3057/96 $15.00 + .00

PII S0091-3057(96)00063-9

Pharmacological Analysis of Diisopropyl Fluorophosphate: Effects on Core Temperature, Heart Rate, and Motor Activity in the Unrestrained Rat

CHRISTOPHER J. GORDON[1]

Neurotoxicology Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Received 19 June 1995; Revised 3 January 1996; Accepted 9 January 1996

GORDON, C. J. *Pharmacological analysis of diisopropyl fluorophosphate: Effects on core temperature, heart rate, and motor activity in the unrestrained rat.* PHARMACOL BIOCHEM BEHAV **55**(2) 185–194, 1996.—Humans acutely exposed to anticholinesterase (anti-ChE) pesticides often become febrile, whereas rats and other rodents become markedly hypothermic. The rat may nonetheless be a useful model for anti-ChE toxicity because recent work using radiotelemetry demonstrated an elevation in core temperature of unrestrained rats for several days following acute exposure to the anti-ChE, diisopropyl fluorophosphate (DFP). To discern the mechanisms of DFP-induced hypothermia and hyperthermia, various pharmacological agents were administered acutely or chronically to rats injected with 1.5 mg/kg DFP (SC). Core temperature, heart rate, and motor activity were monitored continuously via radiotelemetry. Methylscopolamine, a peripheral muscarinic antagonist, attenuated the DFP-induced hypothermia by 1.0°C and reversed the DFP-induced bradycardia. Chronic scopolamine, a central and peripheral muscarinic antagonist, delivered via a subcutaneously implanted minipump (9.5 mg/kg/day) blocked DFP-induced hypothermia and hyperthermia. Propranolol (10 mg/kg; SC), a general beta blocker, augmented the bradycardic effects of DFP but had no effect on body temperature. Sodium salicylate (200 and 300 mg/kg; IP), an antipyretic that inhibits prostaglandin synthesis, administered during the period of DFP-induced hyperthermia produced a transient recovery in body temperature. Overall, DFP-induced hypothermia and hyperthermia in the rat appear to be mediated via cholinergic activation in the CNS because both are blocked by scopolamine. The decrease in core temperature following sodium salicylate suggests that prostaglandin release is involved in the manifestation of DFP-induced hyperthermia. The elevation in core temperature after DFP appears to involve neurochemical pathways similar to that of fever. **Copyright © 1996 Elsevier Science Inc.**

Anticholinesterase Scopolamine Methylscopolamine Propranolol Salicylate Fever Telemetry Hypothermia Hyperthermia

IRREVERSIBLE inhibition of cholinesterase (ChE) activity is a major neurotoxic effect of organophosphate (OP) compounds [for review, see (2)]. ChE inhibition leads to a rapid synaptic accumulation of acetylcholine, resulting in transient cholinergic stimulation. Rodents acutely exposed to OP pesticides and other anticholinesterase (anti-ChE) agents show well-characterized signs of cholinergic stimulation including bradycardia, lacrimation, tremor, and hypothermia (13,19). Interestingly, humans exposed to anti-ChEs also show many of these signs of cholinergic stimulation with one exception; hyperthermia or a fever rather than hypothermia (13,15,22).

Such divergent thermoregulatory responses to anti-ChE agents confounds the use of rodents as experimental models of anti-ChE neurotoxicity in humans. However, application of radiotelemetry procedures in this laboratory to monitor core temperature in unrestrained rats demonstrated a hyperthermic response in the rat treated with the anti-ChE diisopropyl fluorophosphate (DFP), which lasted for several days after the initial period of hypothermia (11,12). Radiotelemetry is advantageous for detecting subtle elevations in core temperature in rodents that probably would not be observed using the usual, albeit stressful technique of inserting a probe into the colon.

This paper has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

[1] To whom requests for reprints should be addressed.

The hyperthermic response of the rat to DFP may be a useful experimental model to understand the mechanisms of anti-ChE–induced fever. There is little information on the neurochemical mechanisms of DFP-induced hypothermia and hyperthermia. By using radiotelemetry to monitor the acute and long-term effects of ChE inhibition, the pharmacological mechanisms of action are more discernible. The purpose of this study was to use cholinergic antagonists and other pharmacological agents to understand the neurochemical events responsible for DFP-induced effects on core temperature, heart rate, and motor activity in the rat.

METHOD

Male rats of the Long–Evans strain were obtained from Charles River Laboratories at 60 days of age and were tested approximately 30 days later. The rats were housed singly in acrylic cages lined with wood shavings and maintained at an ambient temperature (Ta) of 22°C, 50% relative humidity, and 12 L:12 D period.

Surgery

The rats were implanted with radiotelemetry transmitters (Data Sciences, St. Paul, MN) to monitor core temperature, heart rate, and motor activity as described previously (11). Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg; IP). An incision was made along the midline of the abdomen to implant a radiotelemetry transmitter (model TA11-CTA-F40) into the abdominal cavity. The two wire leads from the transmitter were tunneled under the skin and positioned near the upper right quadrant and lower left quadrant of the chest to detect the heart's electrocardiogram. The abdominal wall and skin were sutured and the rat was allowed at least one week recovery before testing.

Fourteen-day osmotic minipumps (Alzet Corp., Palo Alto, CA; model 2002) were implanted in some animals following at least 1 week of recovery from the telemetry surgery. The rats were anesthetized with either metofane or sodium pentobarbital. The interscapular fur was clipped and a small incision was made for insertion of the minipump. After implanting the pump, the incision was closed with one or two wound clips and the rat was returned to the animal quarters.

Rats were housed individually in their home cages throughout the study. Each cage was placed over a receiver to pick up the signal from the telemetry unit. Heart rate, core temperature, and motor activity were recorded at 10-min intervals as described previously (11). The telemetry data were stored on disk and retrieved at a later time for analysis.

Protocol

Each experiment involved administration of 1.5 mg/kg DFP (Sigma, St. Louis, MO) to all rats. Ten rats were normally tested at a time, with half receiving an additional drug treatment while the other half received saline. Drugs were administered either acutely by subcutaneous (SC) or intraperitoneal (IP) injection or chronically via the minipump. The drugs and their general pharmacological mechanism of action used were: methylscopolamine, a muscarinic antagonist that does not cross the blood–brain barrier; scopolamine, a muscarinic antagonist that does cross the blood–brain barrier; propranolol, a general sympathetic beta-receptor antagonist; and sodium salicylate, an inhibitor of cyclo-oxygenase activity.

The following combination of pharmacological agents and DFP were administered: acute methylscopolamine bromide (1.0 mg/kg; SC) coadministered with DFP); acute scopolamine (0.5 mg/kg, SC; coadministered with DFP); acute scopolamine (1.0 mg/kg, SC; administered 4 and 28 h after DFP); acute propranolol (10 mg/kg, SC; coadministered with DFP); chronic scopolamine (9.5 mg/kg/day administered 7 days before and 3 days after DFP); chronic methylscopolamine nitrate (25.6 mg/kg/day, sc; administered 7 days before and 3 days after DFP); chronic sodium salicylate (29.5 mg/kg/day, SC; administered 7 days before and 3 days after DFP); and acute sodium salicylate (200 and 300 mg/kg sodium salicylate, IP; administered 24 and 48 h after DFP). All drugs were obtained from Sigma. Chronic dose calculations were based on the nominal flow rate of the minipump as provided by the manufacturer (0.5 μl/h) and body weight of rat at the time of DFP injection. The doses of drugs used in the chronic experiments were selected on the basis of minipump studies from the literature using similar doses which appear to be effective in modulating behavioral and/or autonomic functions (16,21).

A total of 89 rats were implanted with transmitters. Six animals died as a result of the DFP administration (6.7%) and there was a transmitter failure in one animal. Telemetry data were monitored for at least 3 days after administration of DFP. Some rats were also administered the peanut oil vehicle 96 h prior to DFP to assess their response to handling and injection procedures. Responses to the pharmacological agents were also observed in some animals prior to DFP administration. Mean ± SE body weight for all rats at the time of DFP injection was 449 ± 6 g.

Temperature Clamping

Following the pharmacological studies, additional experiments were designed to discern if DFP-induced hypothermia contributed to the long-term elevation in core temperature. By anesthetizing the rats and maintaining their core temperature at a hypothermic level for a prolonged time period, it was possible to partially mimic the acute hypothermic response in the rat treated with DFP.

Rats were implanted with transmitters that monitored core temperature and motor activity (model TA10TA-F40) and were allowed to recover for at least 1 week. Following a 24-h period of monitoring baseline core temperature and motor activity in the animal facility, a rat was brought to the laboratory at ~0830 h and anesthetized with nembutal (~50 mg/kg; IP). Once the desired plane of anesthesia was reached, a thermistor probe (Yellow Springs Instruments) was inserted 5 to 6 cm into the colon and the probe was secured to the rat's tail with tape. The signal from the probe was fed to a temperature controller (YSI; model 71A), which was set at either 34 or 37.5°C. The rat was placed on top of an aluminum plate, which could be heated via circulation of water through a manifold. Core temperature of the rat was regulated via the temperature controller, which actuated a pump to circulate 42°C water through the aluminum plate. The rat and the aluminum plate were housed inside an environmental chamber that was kept at a cold Ta of ~10°C for animals clamped at 34°C and at a Ta of ~25°C for animals clamped at 37.5°C. Core temperature was recorded via a thermocouple that was secured adjacent to the thermistor probe with silicon adhesive. The signal from the thermocouple was fed to a digital-analog recorder (Dianachart, model DG-5), which automatically recorded core temperature every 8 s.

After anesthesia and placement of the temperature probes, the the desired clamped core temperature was achieved in less than 30 min. To clamp core temperature, maintenance

TABLE 1

SUMMARY OF EFFECTS OF ACUTE AND CHRONIC PHARMACOLOGICAL TREATMENTS ON DFP-INDUCED BRADYCARDIA, HYPOTHERMIA, HYPERTHERMIA, AND HYPOACTIVITY

	DFP-induced response			
Treatment	Bradycardia	Hypothermia	Hyperthermia	Hypoactivity
Methylscopolamine*	attenuated	attenuated	no effect	no effect
Scopolamine*	attenuated	attenuated	no effect	no effect
Propranolol*	accentuated	no effect	no effect	NT
Methylscopolamine†	blocked	no effect	no effect	no effect
Scopolamine†	attenuated	blocked	blocked	attenuated
Sodium salicylate*	NT	no effect	blocked	no effect
Sodium salicylate†	no effect	no effect	no effect	no effect

NT = not tested.
*Acute; †chronic.

doses of nembutal (generally 5 to 10 mg/kg every hour) had to be given to attenuate thermoregulatory reflexes. At approximately 0900 h the rat was administered either peanut oil vehicle or 1.5 mg/kg DFP (SC) and temperature clamping was maintained for 5 h. The actual core temperatures maintained over the 5-h period of temperature clamping were 34.5 ± 0.1 and 37.74 ± 0.1°C for the hypothermic and normothermic groups, respectively. At the end of the clamping period the hypothermic rats were rewarmed to 37°C, a procedure requiring approximately 15 min. The normothermic rats were disconnected from the temperature clamping system with no rewarming. The rats were quickly returned to the animal room and placed in their home cages for monitoring core temperature and motor activity over the next 72 h. To avoid stress-mediated changes in core temperature, care was taken not to disturb the animals during the baseline period to clamping and recovery period. The rats used in this study were 6 months of age at the time of surgery and had a mean ± SE body weight of 583 ± 25 g.

Statistical Analysis

Most of the telemetry data were averaged into 60 min bins to simplify analyses of the long-term responses to DFP. Analyses of the 10 min data recordings were performed on some of the acute drug administrations. The effect of each drug on DFP-induced changes in core temperature, heart rate, and motor activity was assessed separately using a repeated measures two-way analysis of variance (ANOVA; Dynamic Microsystems, Silver Spring, MD). The time course effects of the drugs varied considerably. Therefore, the time interval for application of the statistical procedures was selected after viewing the drug response of each telemetry variable. The temperature clamping data were first analyzed by averaging the core temperature baseline data for 24 h and then calculating the postclamped change in core temperature. This method was useful for illustrating the diurnal elevations in core temperature following DFP administration. The temperature and motor activity data from the temperature clamping study were also analyzed with repeated measures ANOVA.

RESULTS

The time course of DFP's effect on heart rate, core temperature, and motor activity was altered by acute and chronic treatment with the pharmacological agents (Table 1). The time course of the vehicle injection on the telemetry variables is not presented here [see (11)]. The basic response to the peanut oil vehicle is a transient elevation in core temperature, heart rate, and motor activity with a complete recovery within 2 to 3 h after injection. A description of each drug effect in DFP-treated rats is given in the following text and figures.

Acute Methylscopolamine

Methylscopolamine bromide attenuated the bradycardic and hypothermic effects of DFP for approximately 10 h (Fig. 1). DFP's hyperthermic effects were seen during the second and and third days after treatment and were unaffected by methylscopolamine. The dotted line in this and subsequent figures depicts baseline core temperature prior to DFP and illustrates the elevation in core temperature during the light phase after DFP. By the start of the dark phase heart rates of the two treatment groups were similar and began to slowly increase over the next 24 h. The core temperature reduction after DFP was attenuated by ~1.0°C during the first 6 h after DFP. The decrease in motor activity after DFP was unaffected by methylscopolamine. Acute methylscopolamine had no effect on the recovery of the telemetry variables when assessed 24 to 72 h after DFP.

Chronic Methylscopolamine

Chronic treatment with methylscopolamine nitrate (25.6 mg/kg/day) reversed the DFP-induced bradycardia but had little effect on DFP-induced hypothermia (data not presented). The recovery of core temperature was similar for both groups, although the magnitude of the DFP-induced hyperthermia was considerably less in this particular experiment. The reduction in motor activity after DFP was similar in both saline and chronic methylscopolamine groups.

Acute Scopolamine

Scopolamine (0.5 mg/kg) given at the time of DFP led to a rise in heart rate in the first hour followed by a reduction below that of the saline group (Fig. 2a). The initial fall in core temperature after DFP was attenuated by preadministration of scopolamine, an effect that persisted for approximately 6 h after DFP. The recovery of core temperature was unaffected by the scopolamine treatment. That is, both treatment groups became hyperthermic after DFP. DFP's effects on mo-

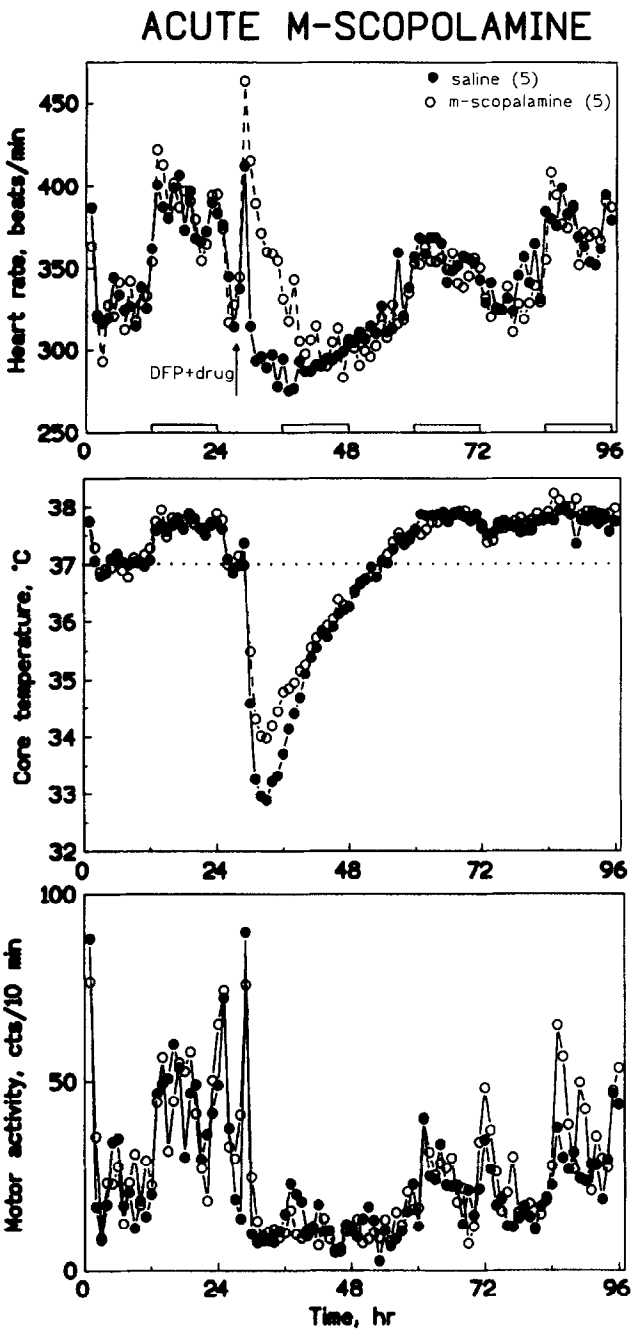


FIG. 1. Effect of 1.0 mg/kg methylscopolamine administered simultaneously with 1.5 mg/kg DFP subcutaneously (sc) on heart rate, core temperature, and motor activity. Arrow indicates time of injection. Repeated measures ANOVA results: heart rate 1 to 10 h after DFP [treatment, $F(1, 8) = 35.9$, $p = 0.0003$; treatment time, $F(9, 72) = 2.5$, $p = 0.01$]; core temperature 2 to 10 h after DFP [treatment, $F(1, 9) = 5.0$, $p = 0.05$; treatment time, NS] treatment time; motor activity 1 to 10 h after DFP (treatment, NS; treatment time, NS). Blocks on abscissa indicate periods of darkness. Numbers in parentheses indicate sample size. Dotted line represents baseline core temperature during light phase.

tor activity were also unaffected by scopolamine treatment. In another acute scopolamine experiment, a 1.0 mg/kg dose of scopolamine given 4 h after DFP led to a rapid but transient recovery in core temperature (Fig. 2b). Administration of 1.0 mg/kg scopolamine 28 h after DFP had little effect on core temperature.

Acute Propranolol

Propranolol (10 mg/kg), given at the same time as DFP, potentiated the reduction in heart rate but had minimal effects on core temperature (Fig. 3). Heart rate after DFP exposure fell to approximately 300 beats/min for 24 h, whereas with propranolol heart rate decreased to as low as 240 beats/min and recovered slowly over the next 15 h. DFP-induced hyperthermia developed in both treatment groups.

Chronic Scopolamine

Chronic scopolamine (9.5 mg/kg/day) was effective in blocking muscarinic receptor function as based on the lack of a typical hypothermic response to the muscarinic agonist oxotremorine (data not presented). The transient elevation in heart rate normally seen after DFP was also absent in the scopolamine treatment group, and there was only a slight increase in heart rate at night relative to the saline treated group (Fig. 4). The core temperature response to DFP was completely blocked by chronic scopolamine. Moreover, the DFP-induced hyperthermia observed 24 to 72 h later was absent in the scopolamine-treated group. Motor activity in the scopolamine group remained elevated over the next ~24 h after DFP. During the second and third nights after DFP exposure, motor activity of the scopolamine group remained elevated relative to that of the saline group.

Chronic Sodium Salicylate

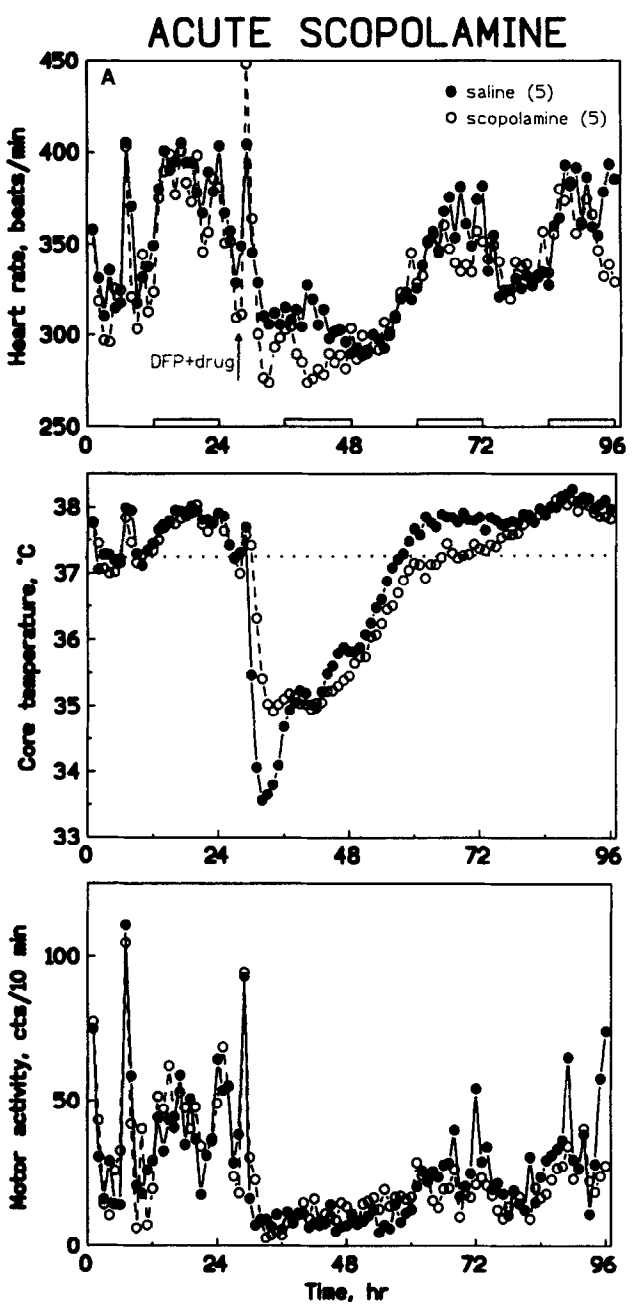
Administration of sodium salicylate at a dose of 29.5 mg/kg/day had no discernible effects on the response to DFP on any of the telemetry variables (data not presented).

Acute Sodium Salicylate

The dose–response study of acute sodium salicylate administration revealed that 300 mg/kg caused a reduction in core temperature below basal levels. A sodium salicylate dose of 200 mg/kg attenuated the stress-induced rise in core temperature but did not lower core temperature below basal levels. The effects of were not distinguishable from that of saline (Fig. 5a). In view of the results from the dose–response study, two separate acute sodium salicylate experiments in DFP-treated rats were undertaken. In one study, saline or 300 mg/kg sodium salicylate was given 24 and 48 h after DFP injection. In another study, saline or 200 mg/kg sodium salicylate was administered once 48 h after DFP (i.e., during the period of DFP-induced hyperthermia). Both the 200 and 300 mg/kg doses of sodium salicylate led to a marked reduction in core temperature followed by a return to the level observed during DFP-induced hyperthermia (Fig. 5b).

Drug Effects

The effects of the pharmacological agents on heart rate, core temperature, and motor activity in untreated rats were also assessed (Fig. 6). Relative to saline, methylscopolamine and scopolamine caused a marked elevation in heart rate. Methylscopolamine had no discernible effect on core temperature and motor activity, whereas scopolamine caused a delayed elevation in core temperature and prolonged elevation in motor activity. Propranolol had little effect on heart rate or core temperature.



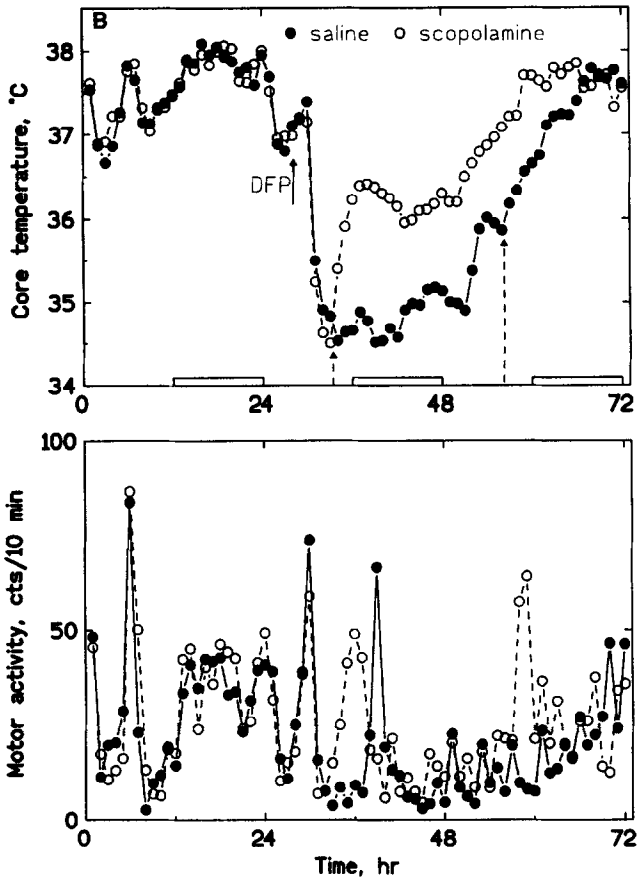


FIG. 2. (a) Effect of 0.5 mg/kg scopolamine (SC) administered simultaneously with 1.5 mg/kg DFP on heart rate, core temperature, and motor activity. Arrow indicates time of injection. Repeated measures ANOVA: heart rate 1 to 16 h after DFP [treatment, NS; treatment time, $F(14, 112) = 3, p = 0.0006$]; core temperature 2 to 5 h after DFP [treatment, $F(1, 8) = 17, p = 0.0033$; treatment time, $F(4, 32) = 6.9, p = 0.0004$]; motor activity 2 to 5 h after DFP (treatment, NS; treatment time, NS). Note cage change on day prior to DFP caused transient elevation in telemetry variables. (b) Effect of 1.0 mg/kg scopolamine (SC) administered 4 and 28 h after 1.5 mg/kg DFP on core temperature and motor activity. Solid arrow indicates time of DFP injection; dashed arrows indicate time of scopolamine injection. Repeated measures ANOVA for first scopolamine injection: core temperature 0 to 15 h after scopolamine [treatment, $F(1, 6) = 8.7, p = 0.02$]; treatment time, $F(15, 90) = 2.4, p = 0.004$]; motor activity 0 to 4 h after scopolamine [treatment, $F(1, 6) = 26.8, p = 0.002$]; treatment time (NS). Second scopolamine response not analyzed. Note cage change on day prior to DFP caused transient elevation in telemetry variables.

Temperature Clamping of Anesthetized Rats

Clamping the anesthetized rat's core temperature at 34.5°C for 5 h had minimal effects on the change in body temperature measured for 48 h after the clamping procedure (Fig. 7). In the hypothermic group given peanut oil there was a slight decrease in core temperature immediately after the cessation of clamping; core temperature rebounded quickly and then appeared to display a normal rhythm by the next day. Normothermic control animals showed a similar response. There was a small, insignificant elevation in core temperature in the normothermic group the day after clamping.

Administration of DFP led to significant changes in body temperature and motor activity during the postclamping recovery period (Fig. 7). Both the normothermic and hypothermic clamped rats underwent a period of hypothermia following termination of the clamping with recovery occuring 12 h later. During the second day after DFP the 24 h mean core temperature was 0.35°C above control in both normothermic and hypothermic animals. Nocturnal motor activity after recovery from clamping was significantly reduced in the normothermic and hypothermic groups given DFP [data not shown; $F(1, 12) = 18.3, p = 0.001$].

DISCUSSION

The major goals of this study were to use a peripheral- and central-acting cholinergic antagonist to explore the mecha-

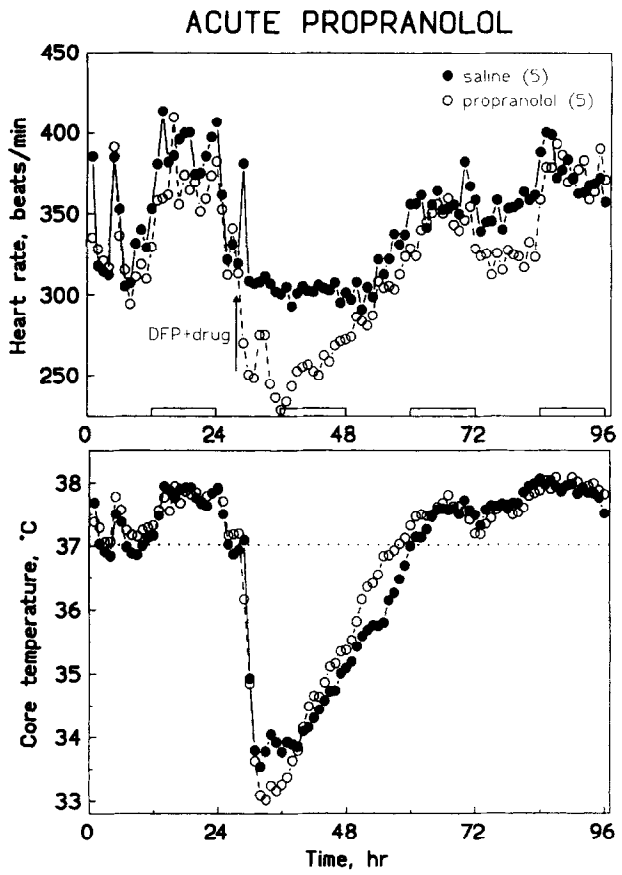


FIG. 3. Effect of 10 mg/kg propranolol (SC) administered simultaneously with 1.5 mg/kg DFP on heart rate and core temperature. Arrow indicates time of injection. Repeated measures ANOVA: heart rate 1 to 9 h after DFP [treatment, $F(1, 8) = 10.5$, $p = 0.01$; treatment time, $F(19, 152) = 2.5$, $p = 0.0009$]; core temperature (treatment, NS; treatment time, NS).

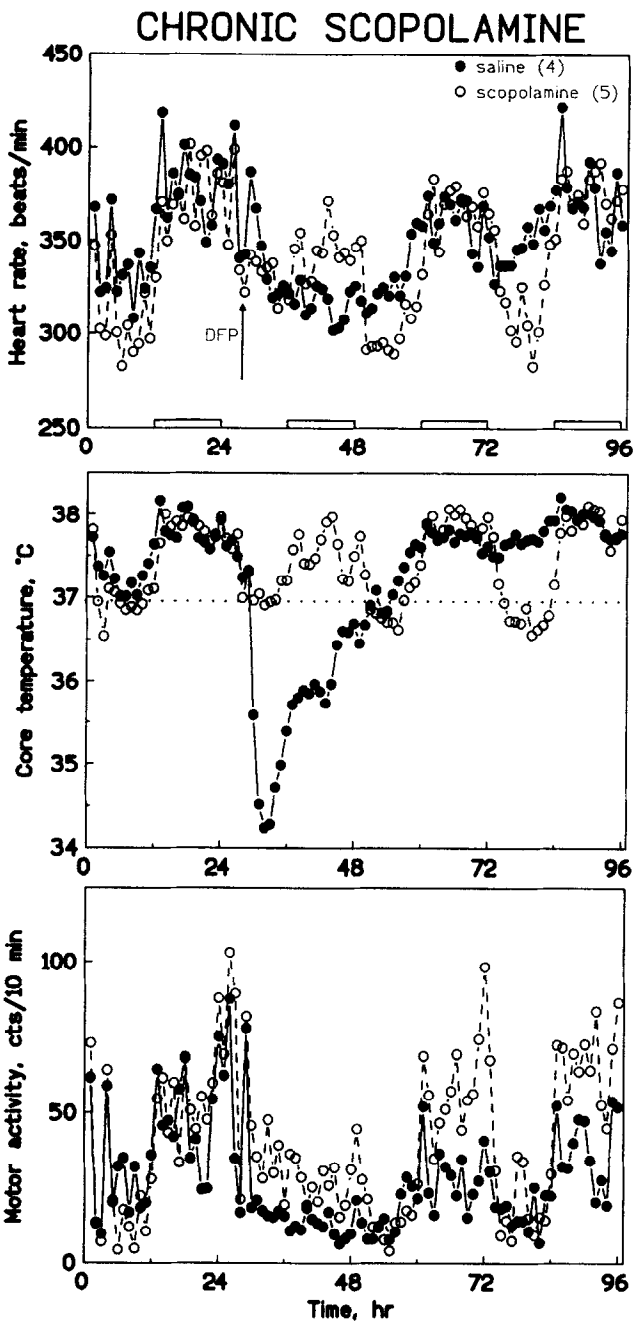


FIG. 4. Effect of chronic scopolamine (9.5 mg/kg/day; SC) on response to 1.5 mg/kg DFP. Arrow indicates time of DFP injection. Repeated measures ANOVA: heart rate 8 to 21 h after DFP (treatment, NS; treatment time, NS); core temperature 1 to 8 h after DFP [treatment, $F(1, 8) = 32$, $p = 0.0005$], treatment time, $F(6, 48) = 49$, $p < 0.0001$]; motor activity 1 to 8 h after DFP [treatment, NS; treatment time, $F(6, 48) = 5.2$, $p = 0.0003$].

nisms of DFP-induced hypothermia and hyperthermia. Methylscopolamine, a peripheral antagonist that does not cross the blood–brain barrier, attenuated DFP's hypothermic efficacy and blocked DFP-induced bradycardia. Scopolamine, a central and peripheral cholinergic antagonist, temporarily reversed DFP's hypothermic effects and had equivocal effects on heart rate. Chronic scopolamine completely blocked DFP's hypothermic and hyperthermic effects. The effects of sodium salicylate, a reversible inhibitor of cyclo-oxygenase, were particularly noteworthy. Administering a dose of sodium salicylate that had no effect on basal core temperature caused a marked recovery of core temperature during the period of DFP-induced hyperthermia. Hence, two key elements are involved in DFP-induced hyperthermia: stimulation of central muscarinic receptors as well as activation of cyclo-oxygenase activity.

Administration of peripheral and central cholinergic antagonists modulated DFP-induced hypothermia in a similar manner seen with past pharmacological analyses of anti-ChE–induced changes in body temperature. Scopolamine attenuated the initial fall in core temperature and also transiently reversed the DFP-induced hypothermia when given several hours after treatment. For example, DFP-induced hypothermia in the rat was partially blocked by administration of 50 mg/kg atropine (IP); however, the same dose of methylatropine, a quaternary antagonist that does not penetrate the blood–brain barrier, had no effect (20). The hypothermic effect of 0.5 mg/kg physostigmine in Sprague–Dawley rats was nearly blocked by the prior administration of either 5.0 mg/kg atropine or 2.0 mg/kg scopolamine, whereas 5.0 mg/kg of methylatropine had

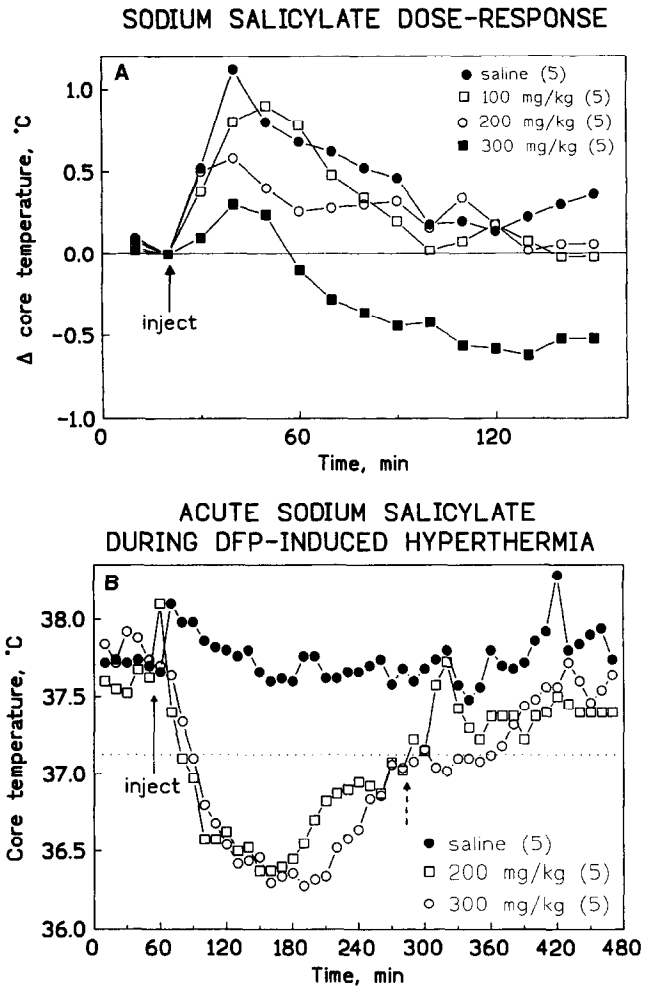


FIG. 5. (A) Dose–response of sodium salicylate (IP) on core temperature of untreated rats. Arrow indicates time of administration. Repeated measures ANOVA for core temperature 10 to 130 min after injection: [treatment, $F(3, 16) = 8.5, p = 0.0013$]; treatment time (NS). (B) Effect of administration of 200 and 300 mg/kg sodium salicylate (IP) on core temperature administered 48 h after development of DFP-induced hyperthermia. Solid arrow indicates time of sodium salicylate injection; dashed arrow indicates time of administration of saline to 200 mg/kg sodium salicylate group. Dotted line denotes basal diurnal core temperature prior to DFP. Repeated measures ANOVA for core temperature 10 to 250 min after injection: [treatment, $F(2, 12) = 7.4, p = 0.0078$; treatment time, $F(48, 288) = 4.3, p < 0.0001$].

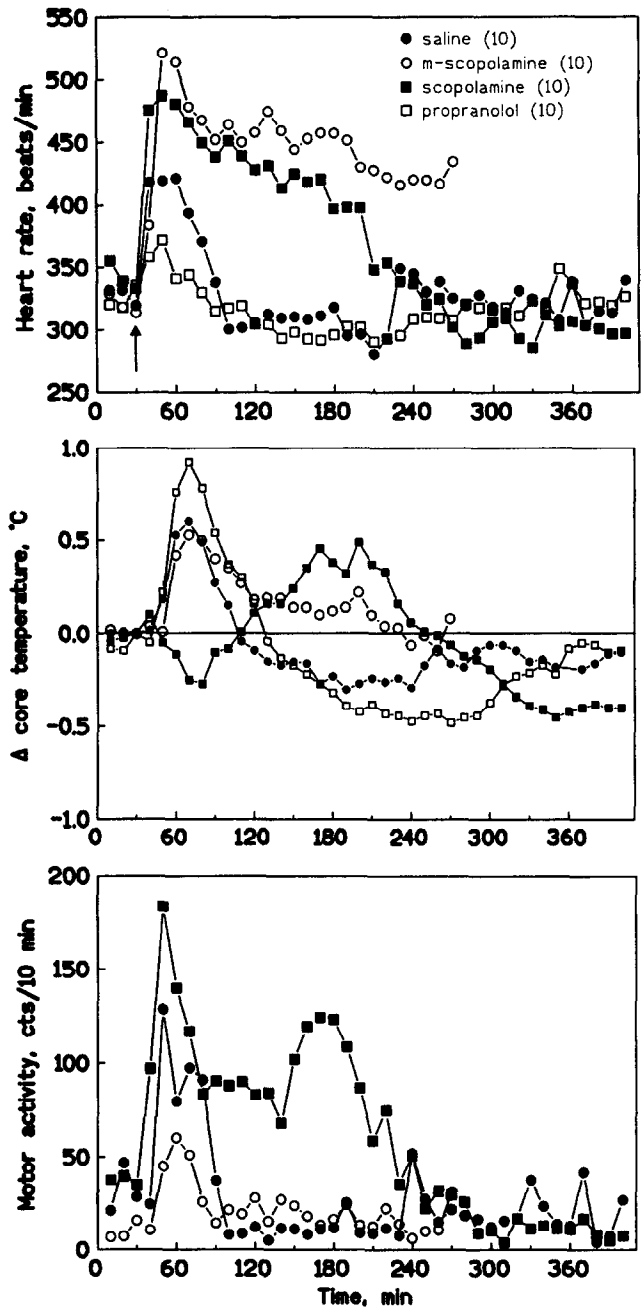


FIG. 6. Time course of heart rate, core temperature, and motor activity of untreated rats administered 0.9% saline (0.1 ml/100 g b.wt.), methylscopolamine (1.0 mg/kg), scopolamine (0.5 mg/kg), or propranolol (10 mg/kg). Arrow indicates time of administration for all drugs. $n = 10$ for each treatment.

no effect (18). Similar antagonistic effects of scopolamine on physostigmine-induced hypothermia have been reported in mice (17). In the present study, methylscopolamine administered acutely caused a slight attenuation in the DFP-induced hypothermia; when administered chronically at a dose to reverse DFP-induced bradycardia, methylscopolamine had little effect on DFP-induced hypothermia. In general, studies on the acute anti-ChE treatment indicate that the hypothermia is mediated primarily through a central activation of muscarinic receptors (M1 presumably) in the CNS (13,25). However, the small but significant effect of methylscopolamine on DFP-induced hypothermia suggests a role for peripheral cholinergic receptors in the control of body temperature following anti-ChE treatment.

The DFP-induced hyperthermia has heretofore not been analyzed using pharmacological tools, and there is little known regarding the mechanisms of anti-ChE–induced hyperthermia. An experimental model is needed because a fever is a common symptom in humans poisoned with anti-ChEs (13,15,22). The initial hypothermic response observed in the rat and other rodents exposed to anti-ChEs is uncharacteristic of human exposures. The relatively large surface area:body mass ratio in rodents facilitates hypothermia, a response that appears to

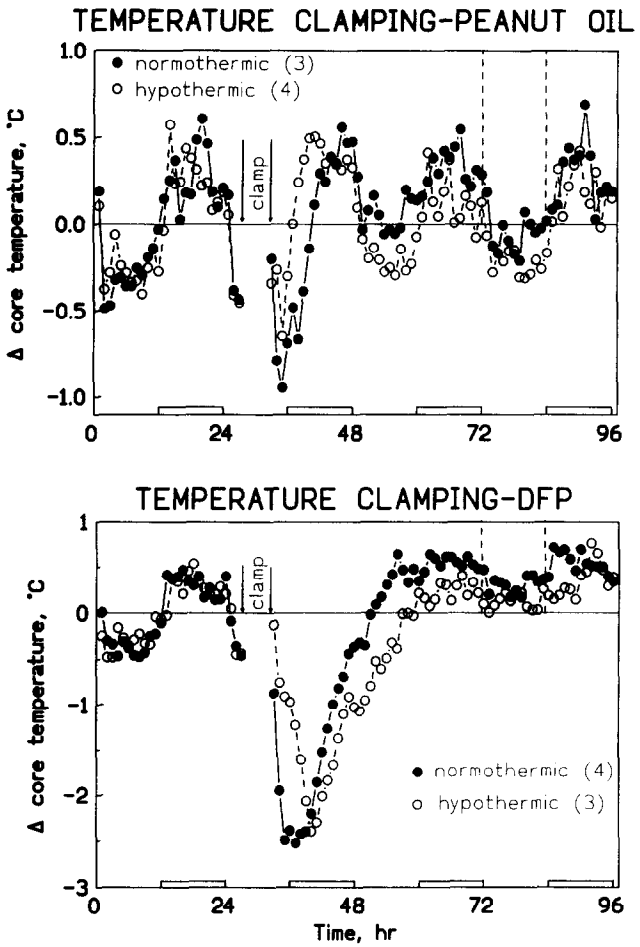


FIG. 7. Time course of change in core temperature of rats subjected to clamping of their core temperature at 34.5 (hypothermic) or 37.7°C (normothermic) for 5 h after administration of the peanut oil vehicle or 1.5 mg/kg DFP. Change in core temperature calculated from 24 h mean of core temperature measured prior to injection. Peanut oil and DFP administered at start of clamping. Three-way repeated measures ANOVA for core temperature during second day after injection (time marked by two vertical dashed lines) (DFP treatment, $F = 7.0$, $p = 0.02$); temperature treatment (NS); temperature–time interaction, $F = 2.8$, $p = 0.002$); DFP–time interaction, $F = 2.7$, $p = 0.003$); DFP–temperature–time interaction (NS).

improve survival to anti-ChEs (13). On the other hand, the delayed hyperthermic response in the DFP-exposed rat may be a useful response for understanding the mechanism of action of the thermoregulatory effects of anti-ChEs in humans. The chronic scopolamine experiment suggests that the DFP-induced hyperthermia is mediated through central activation of muscarinic receptors. Chronic scopolamine also blocked the occurrence of DFP-induced hypothermia. Hence, one would question if the DFP-induced hyperthermia is in some way dependent on the initial period of DFP-induced hypothermia. This is unlikely because anesthetized rats maintained hypothermic for 5 h did not show a delayed elevation in core temperature as was seen in the DFP-treated animals. Rats maintained normothermic at 37.74°C and given DFP showed a hyperthermic response similar to that seen in the unanesthetized animals. Unfortunately, it was not possible to prevent a DFP-induced hypothermia in both the normothermic and hypothermic clamped groups. Nonetheless, when taken together with the response of the hypothermic rats given peanut oil, it would appear that the delayed rise in core temperature seen 48 h after DFP is not attributable to the DFP-mediated hypothermia.

Based on studies with physostigmine, Sen and Bhattacharya (25) proposed that cholinergic hyperthermia is manifested through modulation of M2 muscarinic receptors. Other neurochemical and/or neuroendocrine mechanisms could also be operative. Organophosphates lead to marked changes in central levels of monoamine neurotransmitters, which could be responsible for a hyperthermic response (7). DFP, at a dose as low as 0.6 mg/kg, causes significant elevations in leutenizing hormone and corticosterone 3 h after treatment (27). Although not investigated, such neuroendocrine disturbances could contribute to prolonged changes in body temperature.

Because sodium salicylate is a short-acting inhibitor of cyclo-oxygenase activity, it would appear that DFP-induced hyperthermia is mediated vis-a-vis byproducts of cyclo-oxygenase (8). Sodium salicylate at a dose that normally dose not lower body temperature was effective in lowering the core temperature of rats during the period of DFP-induced hyperthermia. There is no direct link between DFP-induced neurotoxicity and cyclo-oxygenase activity. However, it is known that cholinergic stimulation of brain tissue leads to the release of various prostanoids, including prostaglandin E_2, which is crucial in the development of fever in rodents and other mammals (6,23). Buccafusco et al. (5) found that indomethacin was effective in attenuating the hypothermic-inducing properties of the muscarinic agonist carbachol administered intracerebroventricularly, but only when the indomethacin was administered between two separate injections of carbachol. This may be relevant to the present study because the cholinergic stimulation following DFP would likely activate similar processes as seen with administration of carbachol. A model of muscarinic receptor function proposes that prostaglandin release following muscarinic stimulation leads to enhanced responsiveness of the muscarinic receptors (5).

The thermoregulatory effects of salicylates are somewhat controversial and deserve some attention. In the Sprague–Dawley rat, 300 mg/kg sodium salicylate (IP) had no effect on the diurnal core temperature but did block the nocturnal elevation in core temperature (24). Administration of 5.0 mg of carbachol into the anterior hypothalamus of the rat induced a hyperthermic response that was unaffected by a gavage administration of 24 mg/kg acetylsalicylic acid (1). On the other hand, stress-induced hyperthermia in the rat resulting from placement in an open, well-illuminated field is blocked by pretreatment with 200 mg/kg sodium salicylate (26). Overall, the dose of sodium salicylate effective for blocking the DFP-induced hyperthermia is equivocal to the range of doses reported to block endotoxin fevers, stress-mediated hyperthermia, and nocturnal elevations in core temperature (8,24). The occurrence of DFP-induced hyperthermia with chronic administration of sodium salicylate suggests that therapeutic doses of salicylate are required to block the DFP-induced rise in core temperature. That is, the chronic sodium salicylate dose of 29 mg/kg/day is approximately 50% less that the daily dose needed for analgesia (9).

Cholinergic stimulation of the heart should lead to bradycardia and reduced cardiac output. However, past studies with various anti-ChEs have reported tachycardic, hypertensive responses, as well as bradycardic responses. For example, intracerebroventricular neostigmine causes increased sympathetic nerve activity in various peripheral tissues of the rat, including

heart, liver, and brown adipose tissue (14). Both tachycardic/hypertensive and bradycardic/hypotensive responses in rat have been observed with intravenous administration of organophosphates (3,4). Previous work with DFP in the unrestrained rat showed that the bradycardic effects of DFP were most apparent during the first night after administration, the period when heart rate is elevated in control animals (11). DFP (1.0 mg/kg) led to a mild ~10 beat/min reduction in the diurnal heart rate but a 40–50 beat/min reduction of the nocturnal heart rat. In the present study, the bradycardic effects of DFP during the day and night were exacerbated by the administration of propranolol, a general beta blocker. This suggests that increased sympathetic tone is important in the maintenance of heart rate during cholinergic stimulation arising from DFP treatment.

The present studies have elucidated some of the pharmacological mechanisms of DFP-induced hypothermia, hyperthermia, bradycardia, and motor activity in the rat. The initial hypothermic effects of the anti-ChE on core temperature appear to be mediated primarily through central muscarinic receptor activation and partially through peripheral activation. Interestingly, the long-term effect on core temperature is also mediated through central muscarinic receptors; however, activation of cyclo-oxygenase is crucial for the maintenance of a hyperthermic state following DFP. This finding is novel for anti-ChE mechanisms and could be important to a variety of neurotoxicological studies. For example, these data may help explain the common report of fever seen in humans exposed to anti-ChE pesticides. Also, because prostaglandins and other products of cyclo-oxygenase are critical in the manifestation of many pathologies, these data may open new avenues for studying the mechanisms of anti-ChE toxicity. It should be added that, without telemetry to monitor physiological and behavioral variables in the unrestrained rat, the subtle elevation in body temperature seen several days after the initial DFP exposure would likely not have been observed. Application of radiotelemetric technology with pharmacological tools should be extremely useful in the characterization of the short- and long-term effects of neurotoxicants and other chemical agents.

ACKNOWLEDGEMENTS

I am indebted to Lela Fogelson for her technical assistance. I thank Drs. Amir H. Rezvani and William P. Watkinson for their reviews of the manuscript.

REFERENCES

1. Avery, D. D.; Penn, P. E. Interaction of salicylate and body temperature changes caused by injections of neurohumours into the anterior hypothalamus: Possible mechanisms in salicylate antipyresis. Neuropharmacology 15:433–438; 1976.
2. Ballantyne, B.; Marrs, T. C. Overview of the biological and clinical aspects of organophosphates and carbamates. In: Ballantyne, B.; Marrs, T. C, eds. Clinical and experimental toxicology of organophosphates and carbamates. Oxford: Butterworth-Heinemann; 1992:3–14.
3. Bataillard, A.; Sannajust, F.; Yoccoz, D.; Blanchet, G.; Sentenac-Roumanou, H.; Sassard, J. Cardiovascular consequences of organophosphorus poisoning and of antidotes in conscious unrestrained rats. Pharmacol. Toxicol. 67:27–35; 1990.
4. Brezenoff, H. E.; McGee, J; Knight, V. The hypertensive response to soman and its relation to brain acetylcholinesterase inhibition. Acta Pharmacol. Toxicol. 55:270–277; 1984.
5. Buccafusco, J. J.; Lapp, C. A.; Aronstam, R. S.; Hays, A. C.; Shuster, L. C. Role of prostanoids in the regulation of central cholinergic receptor sensitivity. J. Pharmacol. Exp. Ther. 266:314–322; 1993.
6. Busija, D. W.; Wagerle, L. C.; Pourcyrous, Ml; Leffler, C. W. Acetylcholine dramatically increases prostanoid synthesis in piglet parietal cortex. Brain Res. 439:122–126; 1988.
7. Coudray-Lucas, C.; Prioux-Guyonneau, M.; Sentenac, H.; Cohen, Y.; Wepierre, J. Brain catecholamine metabolism changes and hypothermia in intoxication by anticholinesterase agents. Acta Pharmacol. Toxicol. 52:224–229; 1983.
8. Dascombe, M. J. The pharmacology of fever. Prog. Neurobiol. 25:327–373; 1985.
9. Gabrielsson, J.; Paalzow, L.; Larsson, S.; Blomquist, I. Constant rate of infusion-improvement of tests for teratogenicity and embryotoxicity. Life Sci. 37:2275–2282; 1985.
10. Gordon, C. J. Temperature regulation in laboratory rodents. New York: Cambridge University Press; 1993.
11. Gordon, C. J. Acute and delayed effects of diisopropyl fluorophosphate on body temperature, heart rate, and motor activity in the awake, unrestrained rat. J. Toxicol. Environ. Health. 39:247–260; 1993.
12. Gordon, C. J. 24-Hour control of body temperature in the rat: II. Diisopropyl fluorophosphate-induced hypothermia and hyperthermia. Pharmacol. Biochem. Behav. 49:747–754; 1994.
13. Gordon, C. J. Thermoregulation in laboratory mammals and humans exposed to anticholinesterase agents. Neurotoxicol. Teratol. 16:427–453; 1994.
14. Gotoh, M.; Smythe, G. A. Effects of intracerebro-ventricularly administered neostigmine on sympathetic neural activities of peripheral tissues in rats. Brain Res. 548:326–328; 1991.
15. Hirshberg, A.; Lerman, Y. Clinical problems in organophosphate insecticide poisoning: The use of a computerized information system. Fundam. Appl. Toxicol. 4:S209–S214; 1984.
16. Loullis, C. C.; Dean, R. L.; Lippa, A. S.; Meyerson, L. R.; Beer, B.; Bartus, R. T. Chronic administration of cholinergic agents: Effects on behavior and calmodulin. Pharmacol. Biochem. Behav. 18:601–604; 1983.
17. Maayani, S.; Egozi, Y.; Pinchasi, I.; Sokolovsky, M. On the interaction of drugs with the cholinergic nervous system. V. Characterization of some effects induced by physostigmine in mice: In vivo and in vitro studies. Biochem. Pharmacol. 27:203–211; 1978.
18. Maickel, R. P.; Kinney, D. R.; Ryker, D. L.; Nichols, M. B. Antagonism of physostigmine induced hypothermia and neuroendocrine changes following exposure to different environmental temperatures. Prog. Neuropsychopharmacol. Biol. Psychiatry 15:873–884; 1991.
19. Matthew, C. B. Anticholinergics: Effects on thermoregulation and performance in rats. Neurosci. Biobehav. Rev. 15:141–146; 1991.
20. Meeter, E.; Wolthuis, O. L. The effects of cholinesterase inhibitors on the body temperature of the rat. Eur. J. Pharmacol. 4:18–24; 1968.
21. Morisset, J.; Loiselle, J. Alteration of the rat exocrine pancreas after chronic scopolamine administration. Pharmacology 30:308–313; 1985.
22. Namba, T.; Nolte, C. T.; Jackrel, J.; Grob, D. Poisoning due to organophosphate insecticides: Acute and chronic manifestations. Am. J. Med. 50:475–492; 1971.
23. Reichman, M.; Nen, W.; Hokin, L. E. Acetylcholine releases prostaglandins from brain slices incubated in vitro. J. Neurochem. 49:1216–1221; 1987.
24. Scales, W. E.; Kluger, M. J. Effect of antipyretic drugs on circadian rhythm in body temperature of rats. Am. J. Physiol. 253:R306–R313; 1987.
25. Sen, A. P.; Bhattacharya, S. K. Thermic response of selective

muscarinic agonists and antagonists in rat. Indian J. Exp. Biol. 29:131–135; 1991.
26. Singer, R.; Harker, C. T.; Vander, A. J.; Kluger, M. J. Hyperthermia induced by open-field stress is blocked by salicylate. Physiol. Behav. 36:1179–1182; 1986.
27. Smallridge, R. C.; Carr, F. E.; Fein, H. J. Diisopropylfluorophosphate (DFP) reduces serum prolactin, thyrotopin, luteinizing hormone, and growth hormone and increases adrenocorticotropin and corticosterone in rats: Involvement of dopaminergic and somatostatinergic as well as cholinergic pathways. Toxicol. Appl. Pharmacol. 108:284–295; 1991.