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BRIEF COMMUNICATION

Effects of Scopolamine on Locomotor Activity and Metabolic Rate in Mice

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BUSHNELL, P. J. *Effects of scopolamine on locomotor activity and metabolic rate in mice.* PHARMACOL BIOCHEM BEHAV **26**(1) 195–198, 1987.—Reduction of metabolic rate occurs in rodents in response to intoxication with several chemicals, including amphetamine. In the present study, cholinergic mediation of locomotor activity and metabolic rate was investigated by measuring the effects of scopolamine on the frequency of photobeam breaks, the rate of CO_2 production, and rectal temperature in unrestrained mice. Increasing doses of scopolamine (0, 0.3, 1.0, 3.0, and 10.0 mg/kg IP) increased locomotor activity over a 72-min observation period. CO_2 production (as minute volume exhaled CO_2, $\dot{V}_ECO_2$), measured simultaneously with locomotor activity, was suppressed equally at all doses of the drug. Rectal temperatures taken 72 min after scopolamine declined slightly in a dose-related manner. These results parallel earlier findings with *d*-amphetamine and suggest that divergent effects on metabolic rate and locomotor activity may be induced by centrally-acting compounds acting on more than one neurochemical system.

Scopolamine Locomotor activity Metabolic rate Body temperature Mouse

A growing literature on thermoregulatory effects of drugs and chemicals indicates that changes in metabolic rate may follow intoxication with a variety of compounds. Hypothermic responses in rodents have been documented to soman [20], sulfolane [12,13], chlordimeform [14] and trialkyltins [3, 7, 11]. This hypothermia appears to be regulated, since metabolic rate is suppressed and, when given a choice, the animals choose a relatively cool ambient temperature, thereby maintaining the lowered body temperature [11–14]. Moreover, this hypothermia appears to enhance survival of near-lethal doses of these compounds, since artificially elevating the body temperature increases the mortality rate [13, 14, 20].

Recent studies of metabolic rate and activity levels in mice [2–5] demonstrated the usefulness of CO_2 production, as minute volume CO_2 ($\dot{V}_ECO_2$), in determining the effects of drugs and toxicants on metabolic rate, and showed that changes in metabolic rate may be dissociated from changes in locomotor activity. That is, both toluene inhalation [5] and injection of *d*-amphetamine [2] increased activity and simultaneously suppressed $\dot{V}_ECO_2$. Mechanistic studies [2] indicated that the *d*-amphetamine-induced suppression of $\dot{V}_ECO_2$ was centrally mediated, increased by exercise, and involved more than a change in respiratory quotient. Further, this suppression did not appear to be related to the anorexigenic action of the drug, to stimulation of glucocorticoid secretion, or to activation of naloxone-sensitive opiate receptors. Thus central catecholaminergic stimulation by *d*-amphetamine suppressed metabolic rate while simultaneously increasing locomotor activity.

Since several toxicants induced hypothermia [11–14], and since *d*-amphetamine reduced metabolic rate in mice [2], it may be hypothesized that hypometabolism and hypothermia constitute part of an overall physiological response of the rodent to chemical intoxication. If so, then hypometabolism ought to be observed in response to treatment with compounds acting on a variety of physiological substrates. Of particular interest are compounds that do not simultaneously reduce locomotor activity and metabolism, as is the case with many toxic chemicals. The cholinergic antagonist scopolamine is known to increase locomotor activity in rodents [1,24], thus providing a test compound whose potential hypometabolic effect would not be confounded with reduced locomotor activity. Moreover, since scopolamine acts via cholinergic, rather than catecholaminergic, mechanisms, observation of its metabolic effects would determine whether or not the hypometabolic response is specific to a single neurochemical substrate.

The cholinergic pharmacology of metabolic rate and body temperature is complex, with experimental evidence for suppression of body temperature both by inhibition of acetylcholinesterase [8, 9, 19–21] and by pharmacologic an-

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tagonism of cholinergic transmission [15, 16, 20]. It is not clear how stimulation and inhibition of the same neurochemical system could produce similar physiological responses; however, the similarity of response to both treatments argues in favor of a general hypometabolic response to chemical intoxication. Because the metabolic effects of scopolamine are not well documented, this study undertook to evaluate its effects of this compound on metabolic rate, body temperature, and locomotor activity in mice.

METHOD

Subjects

Sixteen adult male outbred ICR mice (Harlan Sprague-Dawley, Indianapolis, IN), 20–40 g in weight, were housed in groups of four in acrylic cages (13 cm W × 28 cm L × 17 cm H) on pine chip bedding (Beta Chip, Northwestern Products, Warrensburg, NY). Housing rooms provided a 12 hr:12 hr photoperiod with light onset at 6 a.m., ventilation with a one-pass air supply at 12–15 air changes/hr, a temperature of 27±1°C and relative humidity between 45 and 65 percent. Rodent lab chow (Ralston Purina, St. Louis, MO) and water were available ad lib. Animal care conformed to NIH guidelines [23].

Apparatus

The apparatus has been described in detail elsewhere [4]. Briefly, it consisted of eight mouse chambers in an isolation unit, each with an infrared photobeam (Model 1100, Autotron, Danville, IL) to detect locomotor activity. CO_2 concentrations were measured by two infrared CO_2 analyzers (LIRA Model 303, Mine Safety Appliances, Pittsburgh, PA) assorted plumbing, and two integrating chart recorders (Model 252A, Linear Instruments, Reno, NV) interfaced to a PDP8/a computer (PDP8/a, Digital Equipment, Maynard, MA) with SKED system (State Systems, Kalamazoo, MI). Two parallel channels for gas analysis permitted simultaneous measurement of CO_2 concentrations from two chambers; a time-sampling procedure was thus used, in which each chamber was sampled for 1.5 min during each 6-min sampling cycle. Gas flow and pressure were maintained by vacuum pumps and critical orifices at 1.3 l/min and 4–6 cm H_2O vacuum, respectively.

Procedures

Drug administration. Scopolamine hydrobromide (Sigma Chemical, St. Louis, MO) was dissolved in sterile saline at concentrations of 0, 0.075, 0.25, 0.75, and 2.50 mg/ml (as the salt) and injected IP in a dose volume at 0.10 ml/30 g body weight, yielding doses of 0, 0.3, 1.0, 3.0 and 10.0 mg/kg. Each dose was administered to each mouse in a counterbalanced order. No mouse received drug injections more than twice per week. Each mouse was captured, weighed, injected, and placed individually into its test chamber for 72 min without food or water during the light (inactive) phase of the light cycle. Data collection began as soon as the CO_2 concentrations in the first pair of chambers to be sampled had reached a plateau (about 1 min). The system was calibrated daily.

Locomotor activity. Frequencies of photobeam interruptions by the mouse were counted by the computer and their distributions were normalized by square root transformation [22] prior to analysis.

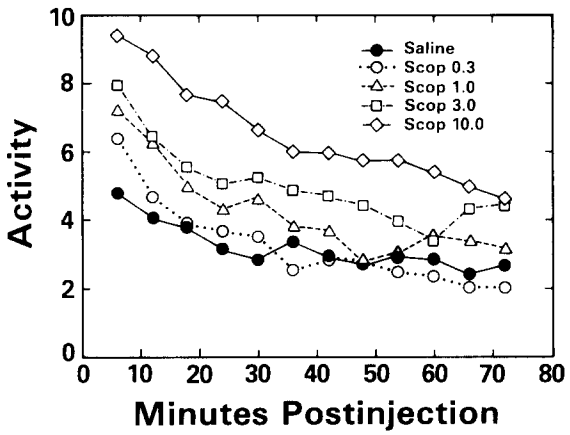

FIG. 1. Locomotor activity of mice (n=8), treated with saline or scopolamine, as a function of time after treatment. Values are mean photobeam interruption frequencies in 6-min time blocks.

$\dot{V}_E CO_2$. CO_2 concentrations in the outflowing air of each mouse chamber were integrated over periods of 1.5 min in each 6-min sampling cycle. Airstream CO_2 concentrations (ml/l) were converted to minute volume expired CO_2 ($\dot{V}_E CO_2$, in ml/min) by multiplication with total airflow (l/min). These volumes were then normalized to the metabolic mass [18] of each animal (body weight in kg raised to the 0.75 power). See [4] for further details.

Rectal temperature. In a separate experiment, 12 additional mice of the same age, strain, sex and housing conditions were treated with doses of scopolamine identical to those used for the other measurements. As before, each mouse received each dose of the drug in a counterbalanced order, with no more than 2 injections given per mouse per week. After injection, each mouse was placed separately in a plastic holding cage of size similar to the metabolic chambers used for determinations of $\dot{V}_E CO_2$. Ambient temperature averaged 24°C. Rectal temperatures were obtained prior to injection and 72 min thereafter using a Vitex telethermometer probe inserted 24 mm into the rectum.

Data analysis. Statistical significance was determined by analysis of variance (BMDP2V [10]), with repeated measures factors for drug and time interval. Post-hoc comparisons between groups were done by simple main effects tests within significant interactions using pooled error terms [17]. The criterion for statistical significance was $p<0.05$ experimentwise.

RESULTS

Scopolamine increased locomotor activity in a dose-related manner (Fig. 1). The overall effect of dose was significant, $F(4,60)=3.82$, $p<0.02$, while the dose by interval interaction was not, $F(44,660)=0.96$. Post-hoc tests of the group means averaged across time intervals showed that doses of 3.0 and 10.0 mg/kg scopolamine significantly increased locomotor activity, while 0.3 and 1.0 mg/kg scopolamine did not.

Conversely, scopolamine suppressed $\dot{V}_E CO_2$ (Fig. 2), as shown by a significant overall effect of dose, $F(4,60)=2.83$, $p<0.04$, and nonsignificant dose by interval interaction,

$F(44,660)=1.08$. Follow-up tests indicated that all doses of scopolamine significantly suppressed $\dot{V}_ECO_2$.

Rectal temperatures were reduced by scopolamine in a dose-related fashion, with (mean±SE) values of 38.4±0.2, 38.1±0.2, 38.1±0.1, 38.1±0.2, and 37.7±0.2°C after saline, 0.3, 1.0, 3.0, and 10.0 mg/kg scopolamine, respectively. Single-*df* comparisons in a one-way repeated measures ANOVA showed that rectal temperature was significantly reduced only after 10 mg/kg scopolamine, $F(1,11)=6.82$, $p<0.03$.

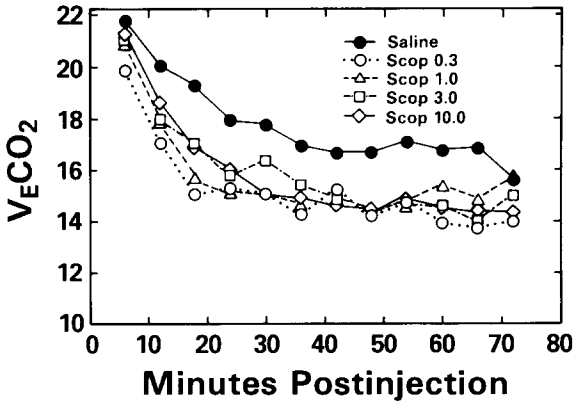

FIG. 2. Metabolic rate of mice (n=8), treated with saline or scopolamine, as a function of time after treatment. Values are mean ml CO_2 exhaled per min per 0.75 kg (minute volume CO_2, V_ECO_2), sampled at 6-min intervals.

DISCUSSION

These data indicate that scopolamine stimulated locomotor activity and suppressed $\dot{V}_ECO_2$ very much like *d*-amphetamine, except regarding the time course of the response. That is, while the stimulation of locomotor activity and suppression of $\dot{V}_ECO_2$ induced by scopolamine did not change in magnitude over the 72-min observation period, the effects of *d*-amphetamine were time-dependent: locomotor activity increased with time, while $\dot{V}_ECO_2$ was most strongly suppressed during the first 30 min after *d*-amphetamine injection, within an effective dose range of 1.0 to 4.5 mg/kg [2]. Thus, despite mediation by different CNS pathways and neurochemistries, both compounds exerted similar divergent effects on locomotor activity and on metabolic rate.

The suppression of metabolic rate by 10 mg/kg scopolamine was sufficient to reduce rectal temperature by 0.7°C. This result is consistent with reports of small changes in rectal temperature after treatment with cholinergic antagonists [6]. Rectal temperatures were not determined after *d*-amphetamine [2].

The fact that the locomotor and metabolic effects of the two compounds diverged indicates that these responses may be dissociated by both scopolamine (Figs. 1 and 2) and *d*-amphetamine [2]. This dissociation in turn suggests that the two responses are produced independently. This argument is supported by differential dose-effect relationships for the two responses: that is, locomotor stimulation increased with increasing dose for both scopolamine (Fig. 1) and *d*-amphetamine [2], while metabolic suppression did not (Fig. 2). Metabolic suppression was in fact induced maximally by both compounds at relatively low doses. The independence of the effects may reflect activation of different pathways, with metabolic rate under autonomic, involuntary control and locomotion under voluntary control.

The ability of scopolamine to suppress $\dot{V}_ECO_2$ is consistent with the reported ability of anticholinergic drugs to reduce metabolic rate in rats [15]. The dissociation of locomotor activity from metabolic rate (as $\dot{V}_ECO_2$) suggests that the hypothermia due to cholinergic suppression must override any heat production resulting from increased locomotor activity. The lack of effect of some cholinolytic hallucinogens on metabolic rate [16] indicates that not all anticholinergic compounds can suppress metabolic rate, despite the clear ability to induce CNS effects.

Nevertheless, the suppression of metabolic rate appears to occur in response to treatment with a number of unrelated compounds including scopolamine, *d*-amphetamine [2], sulfolane [12,13], chlordimeform [14], soman [20], and trialkyltins [3, 7, 11]. Gordon *et al.* [12–14] and Meeter [21] have suggested that hypothermia in response to intoxication may enhance survival by reducing thermodynamically the rate at which the toxicant damages sensitive organs. Perhaps the untoward effects of some drugs, including *d*-amphetamine and scopolamine, are minimized by similar changes in metabolic rate independently of their effects on locomotor activity.

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