ELSEVIER

European Journal of Pharmacology 363 (1998) 107–112

ejp

Sympatho-adrenal involvement in methamphetamine-induced hyperthermia through skeletal muscle hypermetabolism

Toshirou Makisumi [a], Ken-ichi Yoshida [a,*], Tatsuo Watanabe [b], Nobusuke Tan [b], Naotoshi Murakami [b], Akio Morimoto [b]

[a] *Department of Legal Medicine, Yamaguchi University School of Medicine, 1144 Kogushi, Ube, Yamaguchi 755-8505, Japan*
[b] *Department of Physiology, Yamaguchi University School of Medicine, Ube, Japan*

Received 15 September 1998; revised 12 October 1998; accepted 16 October 1998

Abstract

We investigated the involvement of the sympatho-adrenal axis in the hyperthermia induced by methamphetamine by using a biotelemetric system. The intraperitoneal injection of methamphetamine (1 mg/kg) induced hyperthermia preceded by an increase in oxygen consumption in freely moving rats. The hyperthermic effect of methamphetamine was completely blocked by chemical sympathectomy with 6-hydroxydopamine (50 mg/kg, i.p.). Adrenalectomy, but not adrenal demedullation, prevented the hyperthermia. In adrenalectomized rats, dexamethasone supplementation (0.5 mg/kg, s.c.) restored the methamphetamine-induced hyperthermia. Furthermore, dantrolene (1 or 2 mg/kg, i.v.), which blocks Ca^{2+} release from the sarcoplasmic reticulum in skeletal muscle, attenuated the hyperthermia. These results suggest that methamphetamine stimulates norepinephrine release from sympathetic nerve terminals, which then enhances thermogenesis in skeletal muscle under the permissive action of glucocorticoids.

Keywords: Methamphetamine; Hyperthermia; Sympathetic nerve system; Adrenal gland; Glucocorticoid

1. Introduction

Amphetamines have powerful stimulatory actions on the central and peripheral nervous systems and induce hypertension, hyperthermia, and various metabolic derangements (Callaway and Clark, 1994). Amphetamine abuse is a serious social problem, and many abusers have been reported to die as a result of hyperthermia (Askew, 1962; Clark et al., 1967; Zalis et al., 1967). However, the mechanism underlying the hyperthermia induced by methamphetamine is still unknown.

It has not been clarified whether amphetamines induce hyperthermia by acting on the central or the peripheral nervous system. As for the central mechanism, it has been proposed that activation of dopaminergic or serotonergic neurotransmission contributes to hyperthermia (Matsumoto and Griffin, 1971; Frey, 1975; Lin, 1979; Cox et al., 1981; Yamawaki et al., 1983; Gordon et al., 1991). Amphetamine also acts on the preoptic hypothalamic area (Lin et al., 1980). In support of the peripheral mechanism, it has been reported that amphetamine increases core temperature in unrestrained and curarized rats (Borbely et al., 1974) and that it increases the metabolism of brown adipose tissue and induces vasoconstriction through sympathetic activation (Bukowiecki et al., 1982; Astrup et al., 1985; Blaak et al., 1993). However, there have been no experimental studies of skeletal muscle thermogenesis after the administration of amphetamines.

Stress activates the hypothalamo-pituitary-adrenal axis and often occurs in association with amphetamine poisoning. In support of the role of stress, it has been reported that the behavioral and cardiovascular responses elicited by amphetamine administration are influenced by various stressors (Antelman et al., 1980). Additionally, amphetamines elevate the plasma concentration of catecholamines and corticosterone (Knych and Eisenberg, 1979; Vogel et al., 1984). Furthermore, it has been shown that

* Corresponding author. Tel.: +81-836-22-2234; Fax: +81-836-22-2232

0014-2999/98/$ - see front matter
PII: S0014-2999(98)00758-4

sympathetic blockade or adrenalectomy attenuates the behavioral and cardiovascular responses to amphetamine administration (Faunt and Crocker, 1988; Rivet et al., 1989; Maccari et al., 1990; Deroche et al., 1992; Cador et al., 1993; Pauly et al., 1993; Badiani et al., 1995; Johnson et al., 1995). However, there is no report that amphetamines induce hyperthermia through activation of the sympatho-adrenal axis. To examine the latter possibility, it is mandatory to minimize the stress evoked by experimental manipulations. To overcome this difficulty, we used a biotelemetric system to show that the administration of methamphetamine either centrally or peripherally induces cardiovascular, thermal and behavioral responses, and sensitization in freely moving rats (Yoshida et al., 1993). We have also found that the cardiovascular responses elicited by methamphetamine injection are augmented by psychological stress (Makisumi et al., 1995).

In the present study, we focused on the involvement of the sympatho-adrenal system in the hyperthermia induced by methamphetamine by using a biotelemetric system. By means of chemical sympathectomy and adrenalectomy, we found that activation of the sympathetic nervous system and the permissive action of the glucocorticoids are involved in the hyperthermia caused by methamphetamine as a result of hypermetabolism in skeletal muscle.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 300–350 g were used. The rats were housed in individual cages ($40 \times 25 \times 25$ cm) maintained at 26 ± 1°C with a cycle of 12 h light (7:00 a.m.–7:00 p.m.) and 12 h dark. All procedures were reviewed by the committee of Ethics on Animal Experiment in Yamaguchi University School of Medicine and carried out according to the Guidelines for Animal Experiment in Yamaguchi University School of Medicine and Government legislation (Law No. 105 and Notification No. 6).

2.2. Measurement of body temperature and oxygen consumption

Body temperature was measured by using a biotelemetric system (DATAQUEST III, Data Sciences, St. Paul, MN, USA). Rats were implanted intraperitoneally with battery-operated transmitters (model TA10TA-F40) under anesthesia with sodium pentobarbitone (50 mg/kg i.p.) at least seven days before the manipulations. The output of the transmitters (frequency in Hertz) was monitored by antennae mounted in the receiver board (model CTR86) that was placed under each animal's cage. The data were then fed into a peripheral processor (matrix model BCM100) that was connected to a Sanyo MBC-17J AX computer. The details of this telemetric system were described in the previous paper (Lange et al., 1991).

Oxygen consumption was measured with a Beckman oxygen analyzer (model 755), using an open system (airflow: 6 l/min) in a plastic cage ($26 \times 15 \times 12$ cm). The oxygen consumption data were analyzed by a data acquisition control unit (Hewlett-Packard, 3497A), and digital values for each response were printed out every 1 min by a microcomputer printer.

2.3. Chemical sympathectomy, adrenalectomy, and adrenal demedullation

Chemical sympathectomy was produced by i.p. injection of 6-hydroxydopamine (50 mg/kg) 20 h before the injection of methamphetamine. Under these conditions, the peripheral catecholamine level can no longer be measured, but the level in the central nervous system is not affected (Kopin, 1987).

Adrenalectomy or adrenal demedullation was performed one week after the implantation of the biotelemetric transmitter and at least one week before methamphetamine administration. Through the dorsal approach, the adrenal capsule was clamped and the adrenal glands were totally removed for adrenalectomy. For adrenal demedullation, the capsule was clamped and the medulla was extracted through the cortex incision. For sham operation, both adrenals were located and clamped, but not removed. The adrenalectomized rats drank isotonic saline, while the adrenal demedullated and sham-operated rats drank tap water.

To confirm the effectiveness of adrenalectomy and adrenal demedullation and chemical sympathectomy, the plasma concentrations of corticosterone and catecholamines were measured after the experiments. Corticosterone was determined with a radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA, USA), while epinephrine and norepinephrine were measured by High-Performance Liquid Chromatography (Shimadzu, Tokyo). The detection limit was 25 ng/ml for corticosterone, and 5 pg/ml for epinephrine and norepinephrine. Corticosterone and epinephrine were not detected in the adrenalectomized and adrenal-demedullated rats, respectively. In the 6-hydroxydopamine-lesioned rats, neither epinephrine nor norepinephrine was detected.

2.4. Experimental procedure and treatment

After various manipulations, the temporal changes in body temperature were monitored in freely moving rats after the i.p. injection of methamphetamine (1 mg/kg, i.p.). The rats were assigned randomly to six groups: group 1, sham; group 2, chemical sympathectomy with 6-hydroxydopamine; group 3, adrenalectomy; group 4, adrenal

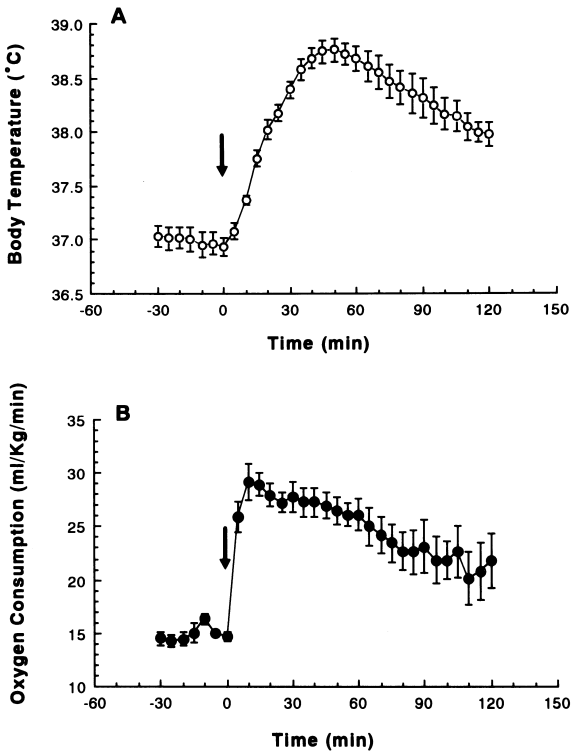

Fig. 1. Changes (mean ± S.E.M.) in the body temperature (A, open circle) and oxygen consumption (B, closed circle) of six rats after methamphetamine (1 mg/kg, i.p.) injection (indicated by the arrow). Methamphetamine increased oxygen consumption and then body temperature.

demedullation; group 5, dexamethasone supplementation to the adrenalectomized rats; group 6, dantrolene. To minimize the confounding effects of the circadian rhythm, all experiments were started between 9:00 and 11:00 a.m.

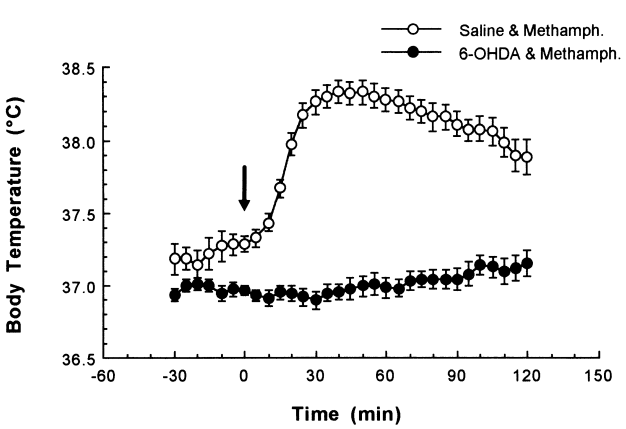

Fig. 2. 6-Hydroxydopamine pretreatment blocked the hyperthermia induced by methamphetamine (1 mg/kg, i.p.). The 6-hydroxydopamine pretreatment (6-OHDA and Methamp.: closed circle, $n = 10$) blunted the metamphetamine-induced hyperthermia (0–120 min; $P < 0.01$) as compared with that of the saline control (Saline and Methamp.: open circle, $n = 6$).

2.5. Drugs and dosage regimen

Methamphetamine (methamphetamine hydrochloride, Dainippon Pharmaceutical, Osaka, Japan) was dissolved in sterile isotonic saline and injected intraperitoneally (i.p., 1 mg/kg body weight). 6-Hydroxydopamine (6-hydroxydopamine hydrobromide, Merck, Wilmington, DE, USA), a specific catecholaminergic neurotoxin, was dissolved in saline containing 0.1% ascorbic acid and injected intraperitoneally (i.p., 50 mg/kg). Dexamethasone (Orgadrone, Sankyo Pharmaceutical, Tokyo, Japan) was dissolved in the saline and injected (0.5 mg/kg) subcutaneously (s.c.). Dantrolene (dantrolene sodium salt, dantrium, Yamanouchi Pharmaceutical, Tokyo, Japan) was dissolved in saline and injected (1 or 2 mg/kg) intravenously. Some adrenalectomized rats were injected with dexamethasone (0.5 mg/kg, s.c.) 1 h before the methamphetamine injection.

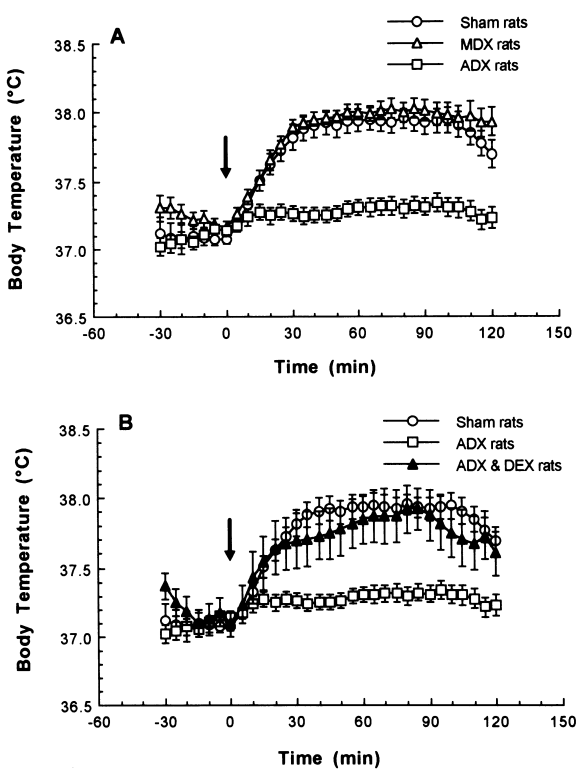

Fig. 3. Effect of adrenalectomy, adrenal demedullation, and dexamethasone supplementation after systemic injection of methamphetamine (1 mg/kg, i.p.). (A) In the adrenalectomized rats (ADX rats: open square, $n = 13$), but not in the adrenal-demedullated rats (MDX rats: open triangle, $n = 9$), the increase in body temperature was significantly lower (0–120 min; $P < 0.01$) than that of sham-operated rats (Sham rats: open circle, $n = 6$). (B) The blockade of methamphetamine-induced hyperthermia in adrenalectomized rats (ADX rats: open squares, $n = 13$) was significantly restored by injection of dexamethasone (0.5 mg/kg, s.c.) in the adrenalectomized rats (ADX and DEX rats: closed triangle, $n = 6$) ($P < 0.01$ at 20–120 min) to the level attained in the sham-operated rats (Sham rats: open circle, $n = 6$).

2.6. Statistical analysis

The data for each time point were analyzed for statistical significance between groups by two-way ANOVA (analysis of variance) followed by a Fisher's post-hoc test.

3. Results

Fig. 1 shows that the body temperature increased rapidly after injection of methamphetamine (1 mg/kg, i.p.), peaking at 50 min, and then gradually declined (panel A). The increase in oxygen consumption preceded that of body temperature with a peak at 10 min after the injection of methamphetamine followed by gradual decline (panel B).

To explore whether the increase in sympathetic outflow is a cause of the hyperthermia, we performed chemical sympathectomy by i.p. injection of 6-hydroxydopamine. As shown in Fig. 2, 6-hydroxydopamine treatment blocked the increase in body temperature induced by the injection of methamphetamine, supporting the sympathetic involvement.

To distinguish between the involvement of the adrenal cortex and the medulla, we compared the temperature response in the adrenalectomized, adrenal-demedullated and sham-operated rats. Fig. 3A shows that adrenalectomy, but not adrenal demedullation, blocked the hyperthermia induced by methamphetamine. As shown in Fig. 3B, the blockage of hyperthermia in the adrenalectomized rats was restored by dexamethasone supplementation, suggesting the involvement of corticosterone.

To investigate whether methamphetamine induces hyperthermia through skeletal muscle hypermetabolism, we examined the effect of dantrolene, which inhibits Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum.

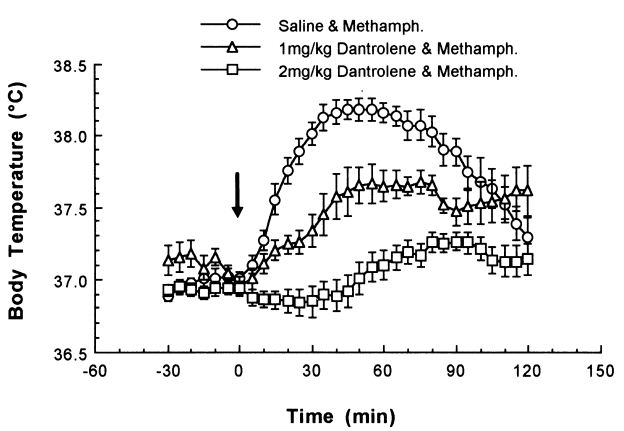

Fig. 4. Dantrolene attenuated the hyperthermia elicited by methamphetamine (1 mg/kg, i.p.). Saline (1 ml/kg, i.v.; open circle, $n = 6$) or dantrolene (1 mg/kg, i.v., open triangle, $n = 6$; 2 mg/kg, i.v., open square, $n = 6$) was injected before methamphetamine (1 mg/kg, i.p.). Dantrolene attenuated the hyperthermia induced by methamphetamine in a dose-dependent manner (saline vs. 1 mg/kg dantrolene, $P < 0.01$ at 15–90 min; saline vs. 2 mg/kg dantrolene, $P < 0.01$ at 10–110 min).

Dantrolene did not affect the basal temperature (data not shown), but, as shown in Fig. 4, it suppressed the hyperthermia induced by methamphetamine in a dose-dependent manner (1 and 2 mg/kg, i.v.).

4. Discussion

The present study is the first to demonstrate the involvement of the sympatho-adrenal axis in methamphetamine-induced hyperthermia, which is caused by skeletal muscle hypermetabolism. This speculation is based on the observation that sympathectomy, adrenalectomy, and dantrolene treatment inhibited the hyperthermic effect of methamphetamine.

Since adrenal demedullation did not suppress methamphetamine-induced hyperthermia (Fig. 3), the suppression of the hyperthermia by 6-hydroxydopamine (Fig. 2) indicates that norepinephrine from the sympathetic nerve terminals, but not epinephrine from the adrenal medulla, contributes to the hyperthermia. It is well-known that corticosterone or other glucocorticoids of the adrenal cortex permit catecholamines or other hormones to induce a number of metabolic reactions (Ganong, 1991). We showed that methamphetamine-induced hyperthermia was blocked in adrenalectomized rats, but was restored by dexamethasone supplementation (Fig. 3). The latter finding suggests that corticosterone or other glucocorticoids from the adrenal cortex permit the hyperthermic effect of norepinephrine released after methamphetamine administration.

The hypermetabolism, as demonstrated by the increase in oxygen consumption, preceded the hyperthermia induced by methamphetamine administration (Fig. 1). This finding suggests that methamphetamine induces metabolic thermogenesis, as has been reported to occur after activation of the sympathetic nervous system by frontal neocortex stimulation (De Luca et al., 1989). Skeletal muscle thermogenesis was previously shown to be induced after administration of ephedrine and other sympathomimetics (Astrup et al., 1985). In this study, we showed that dantrolene blocked the hyperthermic effect of methamphetamine (Fig. 4), suggesting that skeletal muscle thermogenesis contributes to methamphetamine-induced hyperthermia. Dantrolene, an inhibitor of Ca^{2+} release from the sarcoplasmic reticulum, is used to treat patients with malignant hyperthermia, which is caused by hypermetabolism of skeletal muscle due to hyperactivation of Ca^{2+}-induced Ca^{2+} release (Britt, 1984; Ward et al., 1986) or by derangement of catecholamine release (Ohta and Endo, 1986; Haggendal et al., 1988). Furthermore, dantrolene was also shown to be effective in the treatment of hyperthermia due to an overdose of 3,4-methylenedioxymethamphetamine, an amphetamine analogue (Singarajah and Lavies, 1992). Thus, our data give a theoretical basis for the use of dantrolene in the treatment of the hyperthermia induced by

amphetamines. Our preliminary experiment showed that the removal of brown adipose tissue did not affect the methamphetamine-induced hyperthermia (data not shown), although it has been reported that brown adipose tissue is responsible for a considerable portion of metabolic heat production (Foster, 1984; Himmis-Hagen, 1984). Furthermore, dantrolene (2 mg/kg, i.v.) almost completely blocked the methamphetamine-induced hyperthermia (Fig. 4), which suggests that a decrease in heat dissipation due to vasoconstriction does not play a critical role in hyperthermia. Taken together, these observations suggest that the hypermetabolism of skeletal muscle induced by Ca^{2+} release from the sarcoplasmic reticulum is a main cause of the hyperthermia induced by methamphetamine.

Although we focused on the peripheral mechanism of methamphetamine-induced hyperthermia in this study, our data do not contradict the involvement of central mechanisms. The involvement of such mechanisms in methamphetamine-induced hyperthermia is supported by the observations that increased dopaminergic or serotonergic neurotransmission may contribute to methamphetamine-induced hyperthermia, and that dopaminergic activation can induce sympathetic activation (Matsumoto and Griffin, 1971; Frey, 1975; Lin, 1979; Cox et al., 1981; Yamawaki et al., 1983; Gordon et al., 1991).

In conclusion, we found that peripherally administered methamphetamine stimulates the release of norepinephrine from sympathetic nerve terminals, which then enhances heat production in skeletal muscle under the permissive action of glucocorticoids.

Acknowledgements

We are grateful to Dr. Prof. Shoji Nakamura for critical reading of this manuscript and to Dr. Tomoki Nakamori for experimental support.

References

Antelman, S.M., Eichler, A.J., Black, C.A., Kocan, D., 1980. Interchangeability of stress and amphetamine in sensitization. Science 207, 329–331.

Askew, B.M., 1962. Hyperpyrexia as a contributing factor in the toxicity of amphetamine in aggregated mice. Br. J. Pharmacol. 19, 245–257.

Astrup, A., Bulow, J., Madsen, J., Christensen, J., 1985. Contribution of BAT and skeletal muscle to thermogenesis induced by ephedrine in man. Am. J. Physiol. 248, E507–E515.

Badiani, A., Morano, M.I., Aki, H., Robinson, T.E., 1995. Circulating adrenal hormones are not necessary for the development of sensitization to the psychomotor activating effects of amphetamine. Brain Res. 673, 13–24.

Blaak, E.E., Van Baak, M.A., Kempen, K.P.G., Saris, W.H.M., 1993. Role of α and β adrenoceptors in sympathetically mediated thermogenesis. Am. J. Physiol. 264, E11–E17.

Borbely, A.A., Baumann, I.R., Waser, P.G., 1974. Amphetamine and thermoregulation: studies in the unrestrained and curarized rat. Arch. Pharmacol. 281, 327–340.

Britt, B.A., 1984. Dantrolene—review article. Can. Anaesth. Soc. J. 31, 61–75.

Bukowiecki, L., Jahjah, L., Follea, N., 1982. Ephedrine, a potential slimming drug, directly stimulates thermogenesis in brown adipocytes via beta adrenoceptors. Int. J. Obes. 6, 343–350.

Cador, M., Dulluc, J., Mormede, P., 1993. Modulation of the locomotor response to amphetamine by corticosterone. Neuroscience 56, 981–988.

Callaway, C.W., Clark, R.F., 1994. Hyperthermia in psychostimulant overdose. Ann. Emerg. Med. 24, 68–76.

Clark, C.W., Blackman, H.J., Preston, J.E., 1967. Certain factors in aggregated mice D-amphetamine toxicity. Arch. Int. Pharmacodyn. 170, 350–363.

Cox, B., Ennis, C., Lee, T.F., 1981. The function of dopamine receptors in the central thermoregulatory pathways of the rat. Psychopharmacology 20, 1047–1051.

De Luca, B., Monda, M., Amaro, S., Pellicano, M.P., Cioffi, L.A., 1989. Thermogenic changes following frontal neocortex stimulation. Brain Res. Bull. 22, 1003–1007.

Deroche, V., Piazza, P.V., Maccari, S., Lemoal, M., Simon, H., 1992. Repeated corticosterone administration sensitizes the locomotor response to amphetamine. Brain Res. 584, 309–313.

Faunt, J.E., Crocker, A.D., 1988. Adrenocortical hormone status affects responses to dopamine receptor agonists. Eur. J. Pharmacol. 152, 255–261.

Foster, D.O., 1984. Quantitative contribution of brown adipose tissue to overall metabolism. Can. J. Biochem. Cell Biol. 62, 618–622.

Frey, H.H., 1975. Hyperthermia induced by amphetamine, *p*-chloroamphetamine and fenfluramine in rat. Pharmacology 13, 163–176.

Ganong, F.W., 1991. Review of Medical Physiology, 15th edn. Appleton & Lange, East Norwalk, CT.

Gordon, C.J., Watkinson, W.P., O'Callaghan, J.P., Miller, D.B., 1991. Effects of 3,4-methylenedioxymethamphetamine on autonomic thermoregulatory responses of rat. Pharmacol. Biochem. Behav. 38, 339–344.

Haggendal, J., Johnson, L., Johnson, G., Bjurstorm, S., Carlsten, J., 1988. Disordered catecholamine release in pigs susceptible to malignant hyperthermia. Pharmacol. Toxicol. 63, 257–261.

Himmis-Hagen, J., 1984. Non-shivering thermogenesis. Brain Res. Bull. 12, 151–160.

Johnson, D.H., Svensson, A.I., Engel, J.A., Soderpalm, B., 1995. Induction but not expression of behavioral sensitization to nicotine in the rat is dependent on glucocorticoids. Eur. J. Pharmacol. 276, 155–164.

Knych, E.T., Eisenberg, R.M., 1979. Effect of amphetamine on plasma corticosterone in the conscious rat. Neuroendocrinology 29, 110–118.

Kopin, J.I., 1987. Psychopharmacology, The Third Generation of Progress, 1st edn. Raven Press, New York.

Lange, J., Brockway, B., Azan, S., 1991. Telemetric monitoring of laboratory animals: an advanced technique that has come of age. Lab. Anim. 20, 28–34.

Lin, M.T., 1979. Effects of dopaminergic antagonist and agonist on thermoregulation in rabbits. J. Physiol. 293, 217–228.

Lin, M.T., Chandra, A., Chern, Y.F., Tsay, B., 1980. Effects of intracerebroventricular injection of D-amphetamine on metabolic, respiratory, and vasomotor activities and body temperatures in the rats. Can. J. Physiol. Pharmacol. 58, 903–908.

Maccari, S., Lemoal, M., Abgelucci, L., Mormede, P., 1990. Influence of 6-OHDA lesion of central noradrenergic systems on corticosteroid receptors and neuroendocrine responses to stress. Brain Res. 533, 60–65.

Makisumi, T., Yoshida, K., Nakamori, T., Morimoto, A., 1995. Cardiovascular response to methamphetamine is mediated by corticotropin-releasing factor (CRF) and enhanced by stress. Biogenic Amines 11, 247–253.

Matsumoto, C., Griffin, W., 1971. Antagonism of (+)-amphetamine-induced hyperthermia in rats by pimozide. J. Pharm. Pharmacol. 23, 710.

Ohta, T., Endo, M., 1986. Inhibition of calcium-induced calcium release by dantrolene at mammalian body temperature. Proc. Japan Acad. 62, 329–332.

Pauly, J.R., Robinson, S.F., Collins, A.C., 1993. Chronic corticosterone administration enhances behavioral sensitization to amphetamine in mice. Brain Res. 620, 195–202.

Rivet, J.M., Stinus, L., LeMoal, M., Mormede, P., 1989. Behavioral sensitization to amphetamine is dependent on corticosteroid receptor activation. Brain Res. 498, 149–153.

Singarajah, C., Lavies, G.N., 1992. An overdose of ecstasy, a role for dantrolene. Anaesthesia 47, 686–687.

Vogel, W.H., Miller, J., DeTurck, K.H., Routzahn, B.K. Jr., 1984. Effects of psychoactive drugs on plasma catecholamines during stress in rats. Neuropharmacology 23, 1105–1108.

Ward, A., Chaffman, M., Sorkin, E., 1986. Dantrolene. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in malignant hyperthermia, the neuroleptic malignant syndrome and an update of its use in muscle spasticity. Drugs 32, 130–168.

Yamawaki, S., Lai, H., Horita, A., 1983. Dopaminergic and serotonergic mechanisms of thermoregulation: mediation of thermal effects of apomorphine and dopamine. J. Pharmacol. Exp. Ther. 227, 383–388.

Yoshida, K., Morimoto, A., Makisumi, T., Murakami, N., 1993. Cardiovascular, thermal and behavioral sensitization to methamphetamine in freely moving rats. J. Pharmacol. Exp. Ther. 267, 1538–1543.

Zalis, E.G., Lundberg, G.D., Knutson, R.A., 1967. The pathophysiology of acute amphetamine poisoning. J. Pharmacol. Exp. Ther. 158, 115–127.