The effects of indomethacin on the development of tolerance to amphetamine-induced hyperthermia. Are prostaglandins involved?

It is currently accepted that amphetamine exerts many of its pharmacological effects through the release of noradrenaline, although evidence has been produced for a rôle of dopamine in certain effects of this drug (Scheel-Kruger, 1972; Creese & Iversen, 1973). Gessa, Clay & Brodie (1969) have proposed that the hyperthermia produced by amphetamine is a calorigenic effect resulting from the peripheral mobilization of fat from adipose tissue and that this effect requires the release of noradrenaline from sympathetic neurons. Our own studies, in which it was found that the intravenous administration of 6-hydroxydopamine abolished amphetamine-induced hyperthermia, are in accord with the proposal that the elevation in body temperature produced by amphetamine is mediated through the peripheral release of noradrenaline (Trelinski & Sever, in preparation).

In view of the suggestions that prostaglandins of the E group are involved in a negative feedback mechanism controlling the release of noradrenaline from peripheral sympathetic nerves (Smith, 1972), the possibility that noradrenaline-mediated effects of amphetamine could be potentiated by pretreatment of rats with a prostaglandin inhibitor was explored.

Accordingly, female Wistar rats (150–200 g) housed in groups of 5 at constant ambient temperature were dosed with either (+)-amphetamine sulphate (5 mg kg^{-1}, i.p.) alone, or after pretreatment with indomethacin (5 mg kg^{-1} administered by gastric tube) 2 h before amphetamine challenge. Colonic temperature was recorded with a Yellow Springs Instruments Model 46 Thermistor Thermometer using a No. 402 probe, placed 5 cm from the anus, immediately before and at $\frac{1}{2}$, 1 and 2 h after amphetamine injection. The results are shown in Fig. 1. The mean maximal temperature rise evoked by amphetamine after indomethacin pretreatment is signifi-

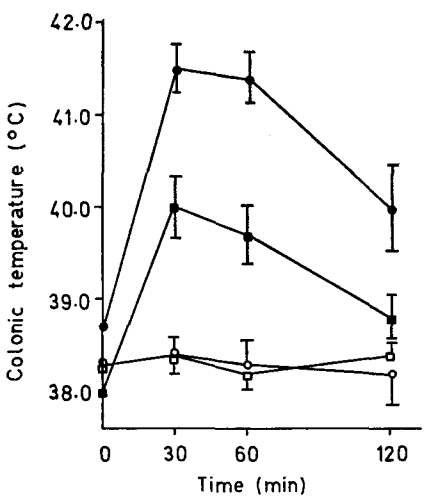

FIG. 1. The effect of indomethacin on the time course of (+)-amphetamine-induced hyperthermia. Rats were pretreated with indomethacin (5 mg kg^{-1} orally) or normal saline (0·5 ml orally) 2 h before injection of (+)-amphetamine (5 mg kg^{-1}, i.p.) or saline. Each curve represents the mean colonic temperature of rats receiving: ● indomethacin before amphetamine (n = 10). ■ Saline before amphetamine (n = 13). ○ Indomethacin before saline (n = 9). □ Saline before saline (n = 6). Vertical bars represent s.e.

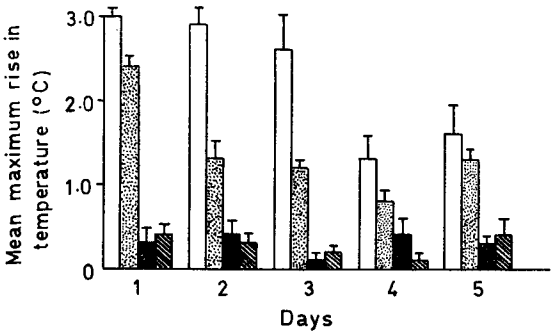

FIG. 2. The effects of indomethacin on the development of tolerance to amphetamine-induced hyperthermia. Rats were treated twice daily with indomethacin (5 mg kg^{-1} orally) or normal saline (0·5 ml) 2 h before injection of (+)-amphetamine (5 mg kg^{-1} i.p.) or normal saline (0·5 ml). Each column represents the mean maximal temperature elevation produced from 0–2 h after the first dose of amphetamine or saline on days 1–5. Open column: indomethacin and amphetamine (n = 8–25). Dotted column: saline and amphetamine (n = 5–8). Solid column; indomethacin and saline (n = 4–9). Hatched column: saline and saline (n = 6). Vertical bars represent s.e.

cantly greater ($P<0{\cdot}05>0{\cdot}01$) than that produced by amphetamine alone. Control experiments confirmed that indomethacin had no effect on baseline temperatures.

Enhancement of the negative feedback mechanism controlling noradrenaline release could be involved in the development of tolerance that is seen with chronic dosage of amphetamines. This was investigated by dosing rats with indomethacin (5 mg kg^{-1}, twice daily) and amphetamine (5 mg kg^{-1}, twice daily) for 5 days. Colonic temperatures were recorded as described, after the first dose of amphetamine on each day. Results are shown in Fig. 2.

From the data presented it is apparent that the tolerance produced to the hyperthermic effect of amphetamine with repeated exposure is partially prevented by indomethacin, a potent inhibitor of prostaglandin synthesis (Ferreira, Moncada & Vane, 1971). Studies with repeated administration of amphetamine alone show that a marked attenuation of the hyperthermic response occurs within 24–36 h, and, although indomethacin prevents this early acquisition of tolerance, on the fourth day of treatment with the combination of drugs tolerance develops.

It could be suggested that indomethacin was producing its effect by inhibiting amphetamine metabolism. To eliminate this possibility, (+)-[^{14}C] amphetamine sulphate (5 mg kg^{-1}, i.p.; 1 μCi per animal) (Smith, Kline and French Laboratories) was administered to rats 2 h after pretreatment with either indomethacin (5 mg kg^{-1} orally) or an equivalent volume of saline orally. The total unchanged amphetamine and metabolites excreted in the first 24 h urine after dosing was determined by solvent extraction and paper chromatography essentially as described by Dring, Smith & Williams (1970) and Caldwell, Dring & Williams (1972). The percentage of dose excreted as amphetamine in two indomethacin pretreated rats was 13 and 18% compared with 15 and 14% in the two saline-pretreated animals. No quantitative difference in the urinary excretion of amphetamine metabolites was found when the two pairs of rats were compared. The recovery of ^{14}C in the 0–24 h urine from the 4 rats varied from 70 to 80%. Thus it would appear that indomethacin does not have an inhibitory effect upon the metabolism of amphetamine.

These results imply that prostaglandin synthesis may be required for the development of tolerance to the hyperthermic effect of amphetamine. Whether two separate mechanisms are involved in amphetamine tolerance, an initial mechanism dependent upon the adequate synthesis of prostaglandins from their precursor fatty acids, and a later mechanism independent of prostaglandins, remains speculative. Lewander

(1971) has suggested that the production of a false transmitter metabolite of amphetamine, *p*-hydroxynorephedrine, which replaced noradrenaline within the sympathetic neuronal vesicles, is liberated on subsequent amphetamine challenge, and is responsible for the attenuation in hyperthermia observed with repeated doses. However, recent studies on sex and species differences in amphetamine metabolism and tolerance militate against the false transmitter hypothesis (Sever & Caldwell, in preparation).

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Inactivation of prostaglandin E_2 by rabbit lung homogenates

Participation of the lung in the biological inactivation of prostaglandins of the E series was first demonstrated by Änggård & Samuelsson (1964) in guinea-pigs. Enzymes present in guinea-pig and swine lungs form three types of metabolites, the most important being the ketone produced by oxidation of the secondary alcohol group at C-15 (Änggård & Samuelsson, 1964, 1965, 1966; Änggård, Gréen & Samuelsson, 1965). Over 90% of the biological activity of PGE_1 was lost after a single passage through the circulation of cat, dog and rabbit lungs (Ferreira & Vane, 1967), or guinea-pigs lungs (Piper, Vane & Wyllie, 1970). Further studies have been made using the purified enzyme (Marrazi & Matschinsky, 1972; Marrazi, Shaw & others, 1972) and lung homogenate (Nakano & Prancan, 1971).

Lung homogenates are easier to use for studying PG inactivation than are the purified enzyme or perfused lungs, and the present work was undertaken to determine the factors which affect this.

Rabbits of either sex were killed by stunning and bled through the carotid arteries. The lungs were rapidly excised and homogenized (Polytron homogenizer) at 4° in four volumes of buffer containing 27·6 mM nicotinamide and adjusted to various pH values (6 to 9). After centrifugation (4° 20 000 *g*, 15 min) the protein concentration in aliquots of supernatant was 9·09 $\pm$ 0·16 (s.e.) mg ml^{-1}, n = 46. In some experiments the supernatant was recentrifuged at 100 000 *g* for 1 h.

PGE_2 (10 μl) was incubated at different temperatures in 1 ml of supernatant containing 0 to 2 mM NAD for 15 to 300 s. The reaction was stopped by adding 2 volumes of absolute ethanol, the mixture was centrifuged (10 000 *g*, 10 min) and the supernatant evaporated to dryness (Evapo-mix). The residue was taken up in 5 ml distilled water, acidified to pH 3·0 with HCl, and extracted twice with two volumes of ethyl acetate. The combined extracts were evaporated to dryness, taken up in 5 ml distilled water, and assayed. Recoveries of PGE_2 added to the incubation medium after ethanol were 88 to 108% (mean: 97·7 $\pm$ 1·2%, n = 23).