(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).

The residual biological activity of the extracts was assayed on rat stomach strips (Vane, 1957) superfused at a rate of 10 ml min^{-1} with Krebs solution at 37° bubbled with 5% carbon dioxide in oxygen, and containing (ng ml^{-1}): methysergide, 2·0; phenoxybenzamine, 1·0; propranolol, 30·0; atropine, 1·0; diphenhydramine, 1·0. Tissues were counterweighted with 1·5 to 2 g, and their movements recorded with isotonic transducers. The strip contracted to amounts of PGE_2 as low as 1 ng ml^{-1}.

Once the tissues had stabilized, a dose-response curve to PGE_2 was obtained. Standards of PGE_2 and extracts were infused at a rate of 1 ml min^{-1} for 3 min into the superfusion fluid, and the responses measured as the areas below the tracing with a Keuffel & Esser planimeter. The standard error of the means and the *t*-test were calculated according to Steel & Torrie (1960).

Drugs: methysergide bimaleate (Sandoz); phenoxybenzamine hydrochloride (Smith, Kline & French); propranolol hydrochloride (Ayerst, McKenna & Harrison); diphenhydramine hydrochloride (Park, Davis & Co.); atropine sulphate (Sigma Chemicals); prostaglandin E_2 (ONO Pharm. Co., Japan).

Influence of pH. When 500 ng of PGE_2 was incubated with 1 ml homogenate in tris buffer containing 2 mM NAD^+, after 30 s of incubation the amount of PGE_2 inactivated at pH 6, 7, 8 and 9, was 29·1 ± 3·0%, 63·0 ± 6·4%, 81·8 ± 3·9%, and 69·0 ± 11·9% respectively (Fig. 1A).

During the next 20 s of incubation, the percentage of inactivation rose to 46·8 ± 6·2, 76·3 ± 0·8, 90·5 ± 1·5, and 77·8 ± 9·1 at pH 6, 7, 8 and 9 respectively. No further inactivation of PGE_2 beyond 90% was seen in the next 4 min at pH 8, whereas the inactivation rose to 80–90% at the other pH values. Additional experiments at pH 7·8 and 8 (n = 5) showed that no further loss of activity occurred up to 60 min incubation. The inactivation of the prostaglandin in the 20 000 and 100 000 *g* supernatants were similar.

Extrapolating the biological half-life of prostaglandin E_2 from the curve obtained in the first 30 s of incubation is difficult. However, additional experiments at pH 8 showed that the percentage of inactivation of PGE_2 after 15 s of incubation was 36·25 ± 1·1%, a value which fits in the curve showed for pH 8 in Fig. 1. Hence, under these conditions, the biological half-life of a dose of 500 ng of PGE_2 would be approximately 18 s.

Influence of temperature (at pH 8·0). When 500 ng PGE_2 in 2 mM NAD^+ was incubated at 22°, the inactivation was decreased to 61·0 ± 16·7% and 77·6 ± 8·0% after 30 ad 60 s respectively, compared to 81·8 ± 3·9% and 90·5 ± 1·5% at 37°. Owing to large variations in the metabolism of the prostaglandin at room temperature, these values were not significantly different (Fig. 1B).

However at 4°, inactivation of PGE_2 was significantly reduced to 9·5 ± 5·4% and 20·7 ± 8·1% respectively at 30 and 60 s ($P < 0.001$ in both cases).

Influence of NAD + (at pH 8; 20 s incubation 37°). With 2·0, 1·0, 0·5, 0·25 and 0 mM NAD^+, PGE_2 500 ng ml^{-1} was inactivated to 63 ± 7·6%, 62·4 ± 7·0%,

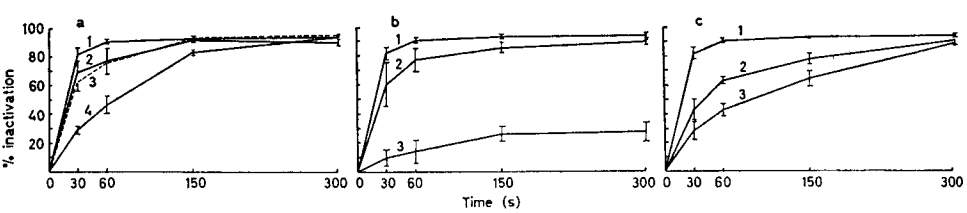

FIG. 1. Effect of (a) pH and (b) temperature on the rate of inactivation of prostaglandin E_2 (500 ng ml^{-1}), and (c) rate of inactivation of prostaglandin E_2 as a function of concentration. Values are mean ± s.e.

59·6 ± 7·5%, 52·6 ± 7·0% and 42·1 ± 5·6% respectively. The inactivation of PGE_2 was significantly reduced with no added NAD^+ compared to that with 1·0 and 2 mM NAD^+ ($P < 0.05$ in both cases).

With increasing doses (0·5, 5 and 10 $\mu g\ ml^{-1}$) of PGE_2 in the presence of 2·0 mM NAD^+, the percentage of inactivation was inversely proportional to the concentration of PG (Fig. 1C). Hence, for the first 30 s of incubation, 81·8 ± 3·9% of a dose of 0·5 μg was inactivated, compared with 43·2 ± 6·9% and 28·0 ± 6·2% for 5 and 10 $\mu g\ ml^{-1}$. However, the absolute amount of PGE_2 metabolized in the first 30 s was proportional to the initial concentration: 13·6, 72·0 and 93·5 ng s^{-1} for doses of 0·5, 5 and 10 μg of PGE_2 respectively. After 5 min of incubation, the three doses of PGE_2 were metabolized to almost the same extent (93·1 ± 0·7%, 90·4 ± 3·4% and 88·7 ± 1·3% with 0·5, 5 and 10 $\mu g\ ml^{-1}$, respectively).

Although changes in pH did not influence significantly the extent to which PGE_2 was metabolized by rabbit lung homogenates after 5 min of incubation, they altered the initial rates of inactivation. Hence, 50% inactivation of PGE_2 was observed after 18 s at pH 8 and 68 s at pH 6. The enzymatic system which converts PGE_2 into inactive 15-keto metabolites has been shown to be NAD^+ dependent (Änggård & Samuelsson, 1964, 1965, 1966), but in our experiments, 40% of PGE_2 was inactivated when PGE_2 was incubated for 20 s without added NAD^+. Moreover, inactivation increased by about 20% when 1 or 2 mM NAD^+ was added. Endogenous NAD^+ might therefore enable some PGE_2 to be metabolized, but the rate of inactivation is significantly increased in the presence of exogenous NAD^+ (1·0 and 2·0 mM). Our studies of pH, temperature and NAD^+ requirements are consistent with the view that inactivation of PGE_2 by rabbit pulmonary tissue is catalysed by a NAD^+ dependent soluble enzyme.

These studies show that the rabbit lung homogenate metabolizes PGE_2 at a rate comparable to that obtained by infusion of prostaglandin into the pulmonary artery *in vivo*. They also show how some of these factors influence the inactivation, and provide a basis for using lung homogenates to investigate prostaglandin metabolism.

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