The present study suggests that the degree of potentiation of the anticoagulant response to warfarin as a result of displacement of bound warfarin by a heavy metal might be increased if persons were occupationally or environmentally exposed for a long time to mercury, cadmium and lead. Although experimental data are not available at the moment, preliminary results from our laboratory, using rats, have demonstrated such a phenomenon. For example, when male Sprague–Dawley rats were pretreated with mercuric chloride (2 mg/kg, i.p.) 24 hr before treatment with warfarin (0.75 mg/kg, i.p.), the prothrombin time was significantly increased from 22 sec in the control to about 49 sec in the treated animal. A significant increase in the anticoagulant response to warfarin was also noticed when both warfarin and mercury were injected simultaneously in rats [10]. At the same time, a diminution of warfarin binding and an initial significant increase in the amount of free warfarin in the plasma of mercury-treated rats were observed *in vivo* (M. Baril, S. Chakrabarti and J. Brodeur, unpublished results). Similarly, pretreatment with $HgCl_2$ (2 mg/kg, s.c.) 24 hr prior to the administration of thiopental (35 mg/kg, i.p.) significantly increased the thiopental sleeping time in rats without affecting hepatic microsomal drug metabolism (S. K. Chakrabarti and J. Brodeur, unpublished results).

Acknowledgements—This research was supported by the MRC Group in Drug Toxicology from the Medical Research Council of Canada. The helpful cooperation of Dr. J. Brodeur is highly appreciated. The expert technical assistance of Mrs. S. Yamaguch and Mr. R. Tardif is gratefully acknowledged.

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Biochemical Pharmacology, Vol 27, pp 2959–2961.
Pergamon Press Ltd. 1978 Printed in Great Britain.

The cumulative effect of halothane and steroids on mitochondrial respiration

(*Received* 18 *September* 1977; *accepted* 11 *April* 1978)

General anaesthetic halothane has been shown to inhibit mitochondrial respiration [1, 2, 3]. The anaesthesia inducing concentration of halothane has been established as 1–1.5 mM *in vivo* and in whole brain tissue [4, 5]; however the effective mitochondrial concentrations of halothane in the course of narcosis remain still unknown. Some experiments performed indicate that mitochondria exposed to gaseous halothane *in vitro* contain about twice as much halothane as these isolated from anaesthetized animals [6]. This clearly indicates that the differences between halothane action in *in vivo* and *in vitro* conditions depend mainly on concentration of this compound. On the other hand, since general anaesthetic properties are attributed to some steroids [7, 8, 9] it was interesting to know whether *in vitro* the presence of such substances would modify effects of halothane. Among steroids investigated progesterone and pregnanolone were found to be a highly potent anaesthetics [8]. It has been also shown that steroids at high concentrations inhibit mitochondrial respiration and oxidative phosphorylation [10, 11].

Placental and cord plasma progesterone concentration has been estimated as 5×10^{-5} M [12]. The aim of the present investigation was to examine the effect of the comparable low steroid concentration on the mitochondrial respiration *in vitro* in the presence of halothane. Evidence is presented in this paper that the presence of progesterone and pregnanolone at low concentrations increases the inhibitory effect of halothane on mitochondrial state 3 respiration. A suggestion is presented that during the halothane induced narcosis, either endogenous or therapeutically administered steroids may potentiate halothane action.

Mitochondria were isolated from rat liver according to the procedure described by Loewenstein *et al.* [13] with omission of the last digitonin step, and resuspended in 0.33 M sucrose with 5 mM Tris–HCl pH 7.3. Protein was determined by biuret reaction in the presence of 1% deoxycholate. Solutions of Analar (Koch–Light) sucrose were passed through the Amberlite IRC–50 resin before use. Tris (Fluka AG) was used after recrystallization from water. Only mitochondria having respiratory control greater than or

equal to 8 were investigated. Each experiment is representative of a number of experiments performed on different mitochondrial preparations and repeated at least 3 times. Halothane (Fluothane Imperial Chemical Industries Ltd., distributed by LEK Ljubliana) dissolved in dimethylformamide (The British Drug Houses Ltd., Poole, England) was introduced into the incubation media in 5 μl portions. Halothane was equilibrated with mitochondrial preparations at 24° and all experiments were performed at the same temperature. Progesterone (Δ^4-Pregnen-3.20-dione, Sigma Chemical Co.) and pregnanolone (5β-Pregnan-3β-ol-20-one, Koch Light Laboratories Ltd., Colnbrook, England) were dissolved in dimethylformamide and added in 5 μl aliquots. The measurements were carried out either in separate vessels or combined vessels allowing simultaneous measurements of both oxygen consumption with Clark oxygen electrode and oxidative phosphorylation by pH changes [14] with combined glass calomel electrode (GR 232 Radiometer with N 512 pH meter Elpo). Vessels were closed in order to avoid the loss of halothane. Both oxygen and pH were registered with two synchronized recorders type I 37/N from Zip.

As may be seen from Table 1 halothane exerted inhibitory effect on glutamate state 3 oxidation which is concentration dependent. Inhibition of state 3 glutamate oxidation amounting to about 33 per cent in the presence of 500 μM halothane alone, increased when low concentrations of either progesterone or pregnanolone had been added (Table 1). It is important to mention that the low concentrations of steroids applied without halothane did not inhibit glutamate oxidation (Table 1, Fig. 1D). Halothane inhibited oxidative phosphorylation measured by the proton uptake and tightly-coupled respiration (Fig. 1 B, C). Progesterone in concentrations which did not affect oxygen and proton uptake (Fig. 1D) appeared to be inhibitory in the presence of halothane (Fig. 1E). When the concentration of progesterone had been increased, a 50 per cent inhibitory effect of this steroid on respiration and oxidative phosphorylation could be observed (Fig. 1F). In the presence of halothane this inhibitory effect reached about 80 per cent (Fig. 1G). The results presented above show that low progesterone and pregnanolone concentrations in the presence of halothane act as inhibitors of NAD dependent substrate oxidation and ATP synthesis. The molecular mechanism of the described above inhibitory effects is not a simple one and requires further investigation. Because observed inhibition may be a joint result of common steroids and halothane action at the level of the membrane it is interesting if these agents possess the same or different site of interaction. The ability of these agents to expand the membrane components was described for steroids [15] and for halothane [16]. The most satisfactory explanation of the expansion phenomena at present is that anaesthetics induce discrete physical changes in membrane proteins when adsorb to hydrophobic regions of proteins [17].

Table 1. The effects of halothane and steroids on state 3 glutamate oxidation.

Additions	Rate of state 3 respiration ng—at 0 (min) mg protein
Control	70
+ Halothane 250 μM	58
+ Halothane 500 μM	47
+ Halothane 1 mM	29
Progesterone 5 μM	70
Progesterone 10 μM	70
Pregnanolone 5 μM	70
Pregnanolone 10 μM	70
Halothane 500 μM	47
+ Progesterone 2 μM	39
+ Progesterone 5 μM	31
+ Progesterone 8 μM	23
+ Progesterone 10 μM	17
+ Pregnanolone 5 μM	28
+ Pregnanolone 10 μM	15

Rat liver mitochondria (5.6 mg of protein in 0.05 ml of 0.33 M sucrose + 5 mM Tris chloride) were added into 3.5 ml of the pH 7.3 media containing: 15 mM KCl, 50 mM Tris chloride, 5 mM $MgSO_4$, 5 mM potassium phosphate and 15 mM potassium glutamate. Mitochondria were preincubated for 2 min with either halothane and steroids while used alone, or with indicated concentrations of steroids and halothane introduced together before the addition of ADP (500 μM).

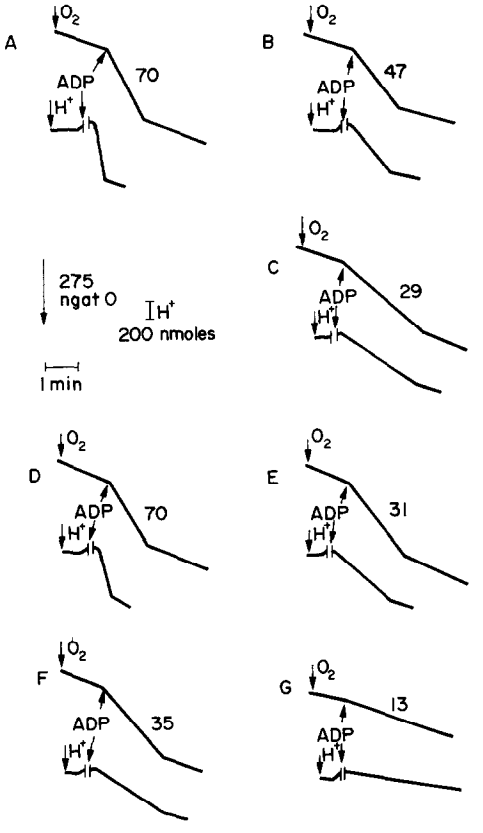

Fig. 1. Glutamate oxidation and oxidative phosphorylation in rat liver mitochondria. The effects of halothane and steroids. Rat liver mitochondria (4 mg of protein in 0.33 M sucrose + 5 mM Tris chloride) were added into 2.5 ml of the pH 7.2 media containing: 200 mM sucrose, 10 mM KCl, 3 mM $MgSO_4$, 5 mM potassium phosphate, 10 mM Tris chloride, 10 μM EGTA, 15 mM potassium glutamate and in exp (B) 500 μM halothane, (C) 1 mM halothane, (D) 5 μM progesterone, (E) 500 μM halothane and 5 μM progesterone, (F) 50 μM progesterone, (G) 50 μM progesterone and 500 μM halothane introduced into the incubation media in the same manner as in Table 1. Oxygen uptake and changes in proton concentration in the medium were recorded simultaneously. Additions: ADP 500 μM. Mark H^+ indicate changes in pH upon addition of 200 nmoles of protons as HCl solution. Differences in oxygen consumption are expressed as ng-atoms O per min and mg of protein. Arrows indicate on oxygen and proton uptake plot respectively.

Acknowledgements—This work was supported by a grant from the Polish Academy of Sciences within the project II. 1.1.6.

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Biochemical Pharmacology, Vol 27, pp 2961–2962
Pergamon Press Ltd 1978 Printed in Great Britain

Effect of prostaglandin A_1, E_2 and $F_{2\alpha}$ on the monoamine oxidase (MAO) activity in rat liver and brain

(*Received* 8 *March* 1978; *accepted* 18 *April* 1978)

Prostaglandins are long-chain unsaturated fatty acids that have been found to be among the biochemical agents that provoke migraine attacks. They have been suggested to be involved in the mechanism of migraine [1]. Intravenous injection of PGE_1 and PGE_2 produces headache of the migraine type [2, 3]. Sandler [4] has postulated that small amounts of prostaglandins released into circulation could account for the vascular phenomenon of migraine by acting on receptors in the cerebral vascular bed. Prostaglandins are potent vasoactive substances probably contributing to the mechanism of pain during migraine in a variety of ways such as by sensitizing the response to other pain-producing stimuli [5] or by direct vasodilation [6]. These observations suggest the modes of action of prostaglandins in provoking the headache. It was thought worthwhile to study the effects of prostaglandins at the neurotransmitter monoamine level. Hence experiments were conducted to study the *in vivo* effect of prostaglandins on the enzyme monoamine oxidase (MAO) (EC 1.4.3.4; monoamine: O_2 oxidoreductase).

Norwegian rats weighing between 250–300 g were used in the present study. Prostaglandins (gift from Upjohn Company, Kalamazoo, Michigan) were first dissolved in ethanol (5 mg/ml) and made up to the required concentration (150 μg in 0.3 ml per injection) with sterile saline. Prostaglandins were injected s.c. at 1100 and 2300 hr everyday for 10 days. PGA_1, PGE_2 and $PGF_{2\alpha}$ were injected into groups of seven animals each. The control group received an equivalent amount of saline under similar treatment. The rats were sacrificed by stunning and decapitation. The liver and brain were removed immediately and homogenized in freshly prepared 0.5 M phosphate buffer pH 7.4. The cell debris including the nucleus was eliminated by centrifuging the crude homogenate in an International Refrigerated Centrifuge at 0° to 4° at 2500 rpm for 15 min. The supernatant was made up to the appropriate volume (20 m/g wt of liver and 40 ml/g wt of brain) with the buffer. This was used as the enzyme preparation for determining the activity of MAO with respect to its different substrates. The enzyme (1 ml) was incubated with 0.4 ml of 0.5 M phosphate buffer pH 7.4 and 1 ml of substrate (600 μg/ml of serotonin or 260 μg/ml of tyramine) for 1 hr, at the end of which period 0.6 ml of 25% TCA was added and chilled immediately to terminate the incubation. Control tubes were pretreated with TCA prior to the addition of the enzyme preparation for determining the activity at zero hour. The tubes were centrifuged and serotonin was estimated in the supernatant by the method of Udenfriend, Weissbach and Clark [7] and tyramine by the method of Udenfriend and Cooper [8]. The pellet was dissolved in 0.1 N NaOH and protein estimated by the method of Sutherland *et al* [9].

The results are shown in Tables 1 and 2. The MAO activity in liver with both tyramine and serotonin as substrates showed an increase with all the three prostaglandins tested, viz., PGA_1, PGE_2 and $PGF_{2\alpha}$. With tyramine as substrate, the increase in activity was highest with PGE_2, it being nearly 70 per cent. When serotonin was used as the substrate, there was an almost equal increase in activity with PGA_1, PGE_2 and $PGF_{2\alpha}$, the increase being approximately 57 per cent. The MAO activity in brain also showed an increase with both the substrates on treatment with PGA_1, PGE_2 and $PGF_{2\alpha}$. The increase with tyramine as substrate was $PGF_{2\alpha} > PGE_2 > PGA_1$. $PGF_{2\alpha}$ treated rats showed a 60 per cent increase in brain MAO activity. With serotonin as substrate, the increase was approximately 54 per cent with