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Electroconvulsive shock raises prostaglandins F in rat cerebral cortex

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The prostaglandins are potent autacoids which are widely distributed in mammalian tissues [1] including the central nervous system of various species [2–4]. Although tissue stores are quite small, prostaglandins (PG's) are rapidly synthesized and released in association with peripheral nerve stimulation [5]. Several groups of workers have reported that PG's and PG-like substances are released from the central nervous system spontaneously and subsequent to electrical or pharmacological stimulation [6–10]. Among these was a report that an unidentified substance, which contracted rat fundus strip in the presence of a serotonin antagonist, was released into the lateral ventricles of cats during convulsions caused by pentylenetetrazol and strychnine [11].

The present study demonstrates striking and rapid increases in cortical levels of PG's of the F series induced by electroconvulsive shock (ECS). The increased level seems to reflect a rapid increase in synthesis. Pretreatment with indomethacin, an inhibitor of PG biosynthesis [12, 13], blocked the increase induced by ECS.

PG's and their metabolites were provided by Dr. J. E. Pike, The Upjohn Co., Kalamazoo, Mich. PG-F_1 [5,6-^{3}H(N)] (sp. act., 67·2 to 89·5 Ci/m-mole) was purchased from New England Nuclear, Boston, Mass. Antisera against PG-F's and against PG-E's were the gift of Drs. L. Speroff and B. Caldwell, New Haven, Conn.

Indomethacin (Merck, Sharp & Dohme, West Point, Pa). was prepared within 1 hr of use by dissolving the powder in alkaline solution and titrating the pH back to 7·4. ECS was delivered to awake animals through gold-plated silver electrodes for 0·2 sec (70–90 V) placed transcorneally or pressed against the scalp biparietally.

Male Sprague–Dawley rats, obtained from Charles River, Inc. and weighing approximately 200 g, were killed by decapitation and their cerebral cortices quickly removed and frozen on dry ice. Frozen tissue was weighed and then homogenized in 0·01 M sodium phosphate buffer, pH 7·4, at 0°. ^{3}H-PG-F_1 was added to an aliquot of homogenate to follow recoveries. The samples were acidified with 1 N HCl and washed with light petroleum ether. PG's were extracted twice into 5 vol. of redistilled ethyl acetate. The dried samples were taken up in benzene–ethyl acetate–methanol (60:40:10) and placed on a 0·5-g silicic acid column. The PG's were separated and eluted by increasingly polar solvent mixtures in discontinuous steps. PG-A's and less polar lipids were eluted with 4 ml benzene–ethyl acetate (60:40), PG-E's with 14 ml benzene–ethyl acetate–methanol (60:40:2), and PG-F's with 4·5 ml benzene–ethyl acetate–methanol (60:40:20) [14]. Dried eluates were taken up in alcohol, diluted and dried again under N_2 prior to radioimmunoassay.

Prostaglandins were not measurably extracted into petroleum ether. Ethyl acetate extracted 80–90 per cent from the acidified aqueous solution. Less than 4 per cent of PG-F was eluted in the PG-E fraction and less than 5 per cent of PG-E was eluted in the PG-F fraction. Overall recovery of PG-F was 65–85 per cent. Recovery was assessed using internal tracer for each sample.

Radioimmunoassay was performed using modifications of the procedure of Caldwell *et al.* [14]. Assay tubes containing 0·1 ml sample, buffer, or standard, 0·1 ml ^{3}H-PG-F_1 (approximately 10^5 cpm) in buffer, and 0·1 ml anti-PG-F antiserum diluted in buffer were incubated at room temperature for at least 1 hr and then cooled to 0°. One ml of freshly prepared dextran-coated charcoal in buffer was added to each sample. After 10 min at 0°, samples were centrifuged and the supernatant containing antibody-bound prostaglandin was decanted into glass scintillation vials. Scintillation fluid was added and the activity of the samples was counted.

Antiserum against PG-F was used at 1:3000 dilution. This gave approximately 50 per cent binding of ^{3}H-PG-F_1. Reagents and their concentrations were as described [14], except that buffers contained 0·01 M sodium phosphate, pH 7·4. Incubation at room temperature followed by adsorption to charcoal at 0° gave more binding and less "stripping" respectively. Antiserum against PG-F was highly specific. The concentration of PG-E_1, PG-E_2, PG-A_1, PG-A_2, 15-keto-PG-F_2, and 13,14-dihydro-15-keto-PG-F_2 required to inhibit binding of ^{3}H-PG-F_1 by 50 per cent was, in each case, greater than 100 times the concentration of PG-F_1 or PG-F_2 required. The antiserum cannot, however, be used to distinguish PG-F_1 from PG-F_2. Standard curves using 0·05 to 2 ng PG-F_1 were run with each assay. Correlation coefficients were reliably above 0·994.

Cortical levels of PG-F's in untreated rats averaged 99·1 ng/g wet weight (PG-F_1 equivalents) with an S.D. for groups assayed on different days of 13·5 ng/g. This is similar to the concentration found in canine cortex [4], though higher than that found in the cortex of cats and chickens [3]. Levels of PG-E in rat cortex were more variable and lower (11 ± 7·5 ng/g).

Preliminary experiments with various centrally acting drugs, administered in pharmacological doses, including *d*-amphetamine (10 mg/kg, i.p.), chlorimipramine (20 mg/kg, i.p.), phenoxybenzamine (25 mg/kg, i.p.), chlorpromazine (10 mg/kg, i.p.), haloperidol (1 mg/kg, i.p.) and lysergic acid diethylamide (0·2 mg/kg, i.p.), failed to show marked changes in the levels of cortical PG's. Pentylenetetrazole (50 mg/kg i.p.), however, did markedly increase the levels of PG-F and PG-E, in association with convulsions.

Electroconvulsive shock induced striking and rapid increases in cortical levels of PG-F's (Fig. 1). Levels were significantly increased within 2 min and peaked approximately 5 min after ECS. ECS induced a 2- to 5-fold increase in PG-F levels. Within 30 min of ECS, PG-F had returned to baseline levels. In view of the evidence for synthesis and release of PG's associated with nervous stimulation in peripheral systems [5] and for release of PG's from superfused cortex associated with electroencephalographic arousal [7], it would seem that the increase induced by ECS may be directly related to increased neuronal discharge.

Indomethacin blocks the synthesis of PG's [12, 13]. Administered to rats (10 mg/kg, i.v.), this drug rapidly reduces the level of PG-F in cerebral cortex (Fig. 2). Pretreatment with indomethacin (20 mg/kg, i.p.) completely blocks the increase in PG-F induced by ECS (Fig. 3). Indomethacin at this dosage, however, does not block the ECS-induced convulsions. This indicates that the increased level of PG-F after ECS reflects a marked stimulation of PG synthesis associated with seizure activity. Since the convulsions, which last about 1 min, ended well before the peak

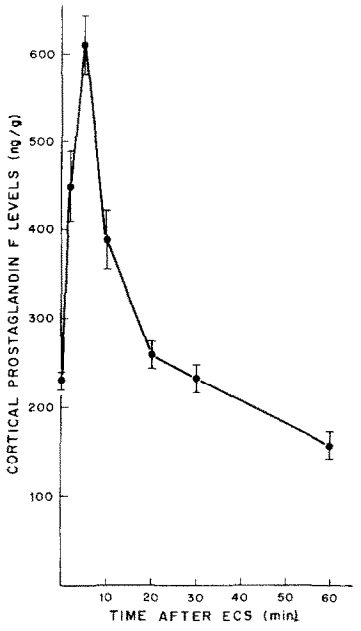

Fig. 1. Effect of ECS on PG-F levels in rat cortex. Rats (350–450 g) were given ECS and killed at the times indicated. The control rats were shocked several times across the rump. Each point is the mean ± S. E. M. of four experiments.

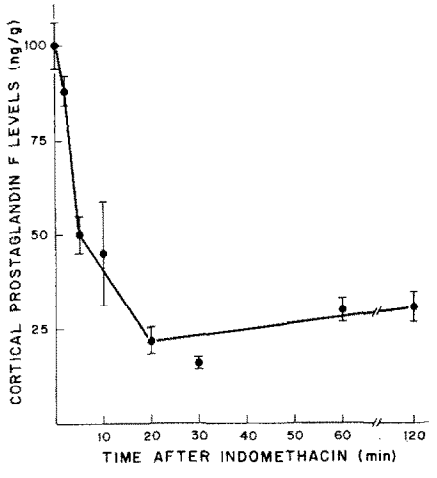

Fig. 2. Effect of indomethacin on PG-F levels in rat cortex. Rats (250–300 g) were injected with indomethacin (10 mg/kg, i.v.) and killed at the times indicated. Each point is the mean ± S. E. M. of four experiments.

of PG levels, it seems unlikely that the convulsions result from an ECS-induced rise in PG levels. In view of the relatively slow metabolism [15] and uptake [16] of PG's by brain tissue, the rapid decline of PG-F levels observed after indomethacin administration and after the initial rise induced by ECS suggests that PG's released by brain tissue may be rapidly cleared into the circulation.

Whether PG's in brain are associated with the actions of particular neuro-transmitters is unclear. In peripheral

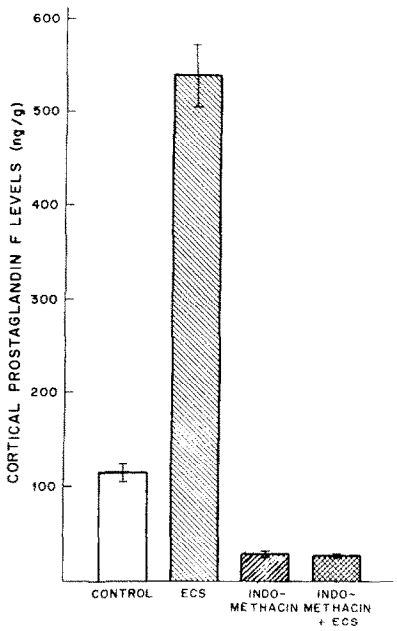

Fig. 3. Effect of indomethacin and ECS on PG-F levels in rat cortex. Rats (250–300 g) given ECS were killed after 5 min. Animals given indomethacin (20 mg/kg, i.p.) were killed after 30 min. Animals given indomethacin + ECS were given ECS 25 min after indomethacin and killed 5 min later. Control animals were injected with saline and shocked across the rump. Mean ± S. E. M. of four experiments.

systems, the release of PG-E occurs during sympathetic nerve stimulation, and it has been suggested that PG's may play a role in modulating noradrenaline release [17]. In the CNS, perfusion of canine lateral ventricles with noradrenaline had no effect on PG release, whereas perfusion with serotonin increased the release of PG-E into the ventricles 4-fold [9]. ECS induces a marked rise in brain serotonin levels [18]. On the other hand, administration of PG-E increased the turnover of serotonin in rat brain [19]. These data suggest the possibility of a relationship between the rise of PG's after ECS and brain serotonin metabolism.

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Effect of acute and chronic iprindole on serotonin turnover in mouse brain*

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Iprindole, 5-(3-dimethylaminopropyl)-6, 7, 8, 9, 10, 11-hexahydro-5H-cyclooct [b] indole hydrochloride (WY-3263), a tricyclic compound, has been shown to be an effective clinical antidepressant agent [1–6]. It was further reported that it has a very weak inhibitory effect on the uptake of norepinephrine, dopamine and serotonin in rat brain tissue [7–10]. It also failed to alter the serotonin content of human platelets [11, 12]. Recently it has been reported that iprindole, after acute or chronic administration, had no effect on rat brain norepinephrine metabolism [13]. Clinically, it has been found to be a more potent antidepressant than imipramine [3–5]. All studies on its mode of action indicate that the mechanism of antidepressant effect of iprindole is not related to its ability to inhibit the neuronal uptake of biogenic amines.

It is well established that all clinically useful antidepressant agents require several days of administration before their beneficial effects are manifest. With this view in mind, we have investigated the effect of acute and chronic administration of iprindole on the turnover of serotonin (5-HT) in mouse brain.

Swiss–Webster male mice weighing 20–22 g were housed five in a cage at a controlled temperature of 22° and constant humidity. The light cycle was set at 12 hr of dark and 12 hr of bright light. The animals had free access to food and water. In acute experiments, one group of mice was given iprindole, 20 mg/kg intraperitoneally, and another group received an equivalent volume of 0·2 ml saline by the same route. In chronic experiments, iprindole (20 mg/kg i.p.) or saline was administered once daily for 3 weeks.

Turnover rate of 5-HT was determined by measuring the decline in 5-HIAA levels and the accumulation of 5-HT at various time intervals after pargyline (75 mg/kg, i.p.) according to the method of Tozer *et al.* [14]. Brain 5-HT and 5-HIAA were determined by the fluorometric procedures of Kuntzman *et al.* [15] and Curzon and Green [16] respectively. The values for brain 5-HIAA levels were logarithmically transformed for calculation of linearity of regression, standard error of the regression coefficients and significance of the difference between regression coefficients [17]. The values for 5-HT were statistically analyzed in the same manner without the logarithmic transformation.

Iprindole, in a dose of 20 mg/kg i.p., administered acutely or chronically for 3 weeks had no significant effect on the rate of 5-HT synthesis or turnover time calculated from the rate of decline in 5-HIAA levels after pargyline (Fig. 1, Table 1), although it slightly increased the rate of 5-HT synthesis and caused a small reduction in its turnover time (Table 1). There was no significant effect of iprindole, whether given acutely or chronically, on the rate of 5-HT accumulation after pargyline (Fig. 2).

The methods used in this study to measure the turnover of 5-HT are based on certain assumptions such as complete inhibition of monoamine oxidase, inability of 5-HT to pass from brain to blood at an appreciable rate, the main catabolic pathway for 5-HT is oxidative deamination and lastly that MAO inhibitor does not affect 5-HT metabolism other than by blockade of MAO. Based on these assumptions, at steady state, the level of brain 5-HT is constant, as a result of equal rates of synthesis and efflux. Similarly the level of 5-HIAA is constant because of equal rates of formation and loss [14]. Data presented here sug-

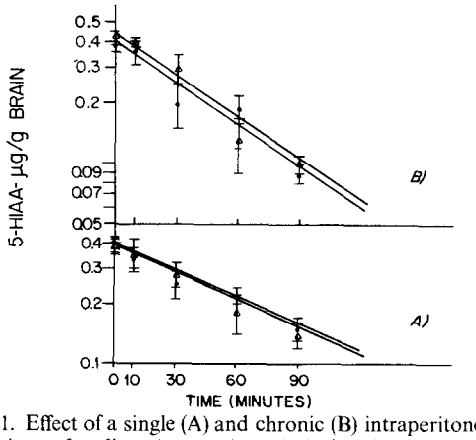

Fig. 1. Effect of a single (A) and chronic (B) intraperitoneal injection of saline (●----●) and iprindole 20 mg/kg (△----△) on 5-HIAA decline in mouse brain at various times after pargyline, 75 mg/kp i.p. Vertical bars represent the standard error of the mean. In Figs. 1 and 2, the method of least squares was used to calculate the best fit line.

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