complexes with lidocaine has been noted using compound 48/80 as a histamine releaser [8].

However, when histamine was released by the ionophores, the inhibitory action of the coordination complex was more pronounced [8] possibly indicating differences in the mechanism of histamine release by antigen or 48/80 on one hand and by ionophores on the other.

Investigated compounds were introduced into a physiological medium (pH 7.0) containing an excess of Cl^- ions. In these conditions Zn(II) ions in both complexes act presumably as $(ZnCl_4)^{2-}$ anions and lidocaine is a protonated form; hence a stronger inhibitory effect than that of lidocaine or zinc alone [7] is obtained.

Considering the inhibitory effect of complexes against broad spectrum of mast cell secretagogues (antigen, compound 48/80, A 23187, X 537A) their interaction with releasing agents seems to be unlikely. Alternatively they may act on mast cells to protect them against various releasers by stabilizing cell membranes. This cromoglycate-type mechanism of action possibly could be helpful in treating allergic disorders.

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The effect of chlorpromazine and 6-hydroxydopamine on arachidonic acid metabolism *in vitro*

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The role of free radicals in prostaglandin (PG) biosynthesis has not been elucidated. It was described that free radicals are inhibitors of cyclo-oxygenase [1–4] since some compounds acting as free radical scavengers are stimulators of prostaglandin generation. On the other hand chlorpromazine, which is also a free radical scavenger [5] has been found to inhibit PG biosynthesis [6–9]. In order to elucidate the role of free radicals in PG biosynthesis we compared the influence of chlorpromazine on this synthesis with that of 6-hydroxydopamine, a known free radical generator [5]. All the methods used have been described previously [10]. The only difference was that unlabeled standards of PGE_2, $PGF_{2\alpha}$ and 6-keto

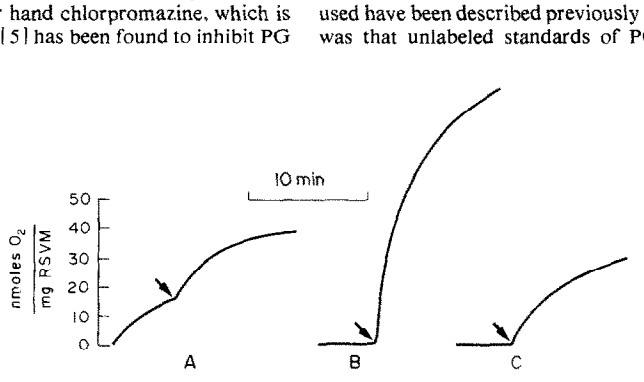

Fig. 1. Tracings of a typical experiment on the influence of chlorpromazine and 6-hydroxydopamine on oxygen consumption by ram seminal vesicle microsomes (RSVM). A. Control incubation mixture containing 1.5 mg microsomes/ml in 0.1M-Tris buffer pH 8.2 and sodium arachidonate (added as indicated by arrow) at the final concentration of 100 μM. B. The same as A + 1000 μM of chlorpromazine added at the beginning of the experiment. C. The same as A + 100 μM of 6-hydroxydopamine added at the beginning of the experiment.

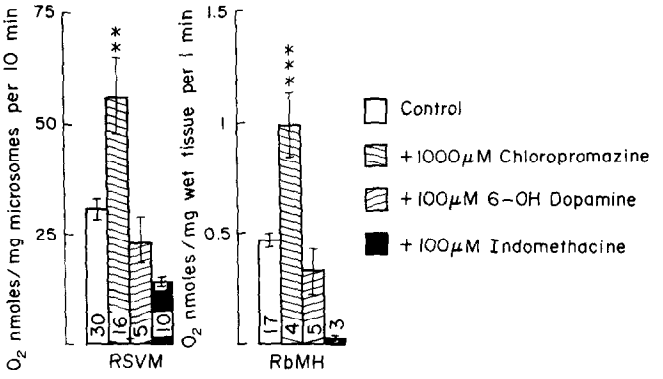

Fig. 2. Oxygen consumption by ram seminal vesicle microsomes (RSVM) and rabbit kidney medulla homogenates (RbMH) in control and treated samples. Experimental conditions were the same as in Fig. 1 and Fig. 4

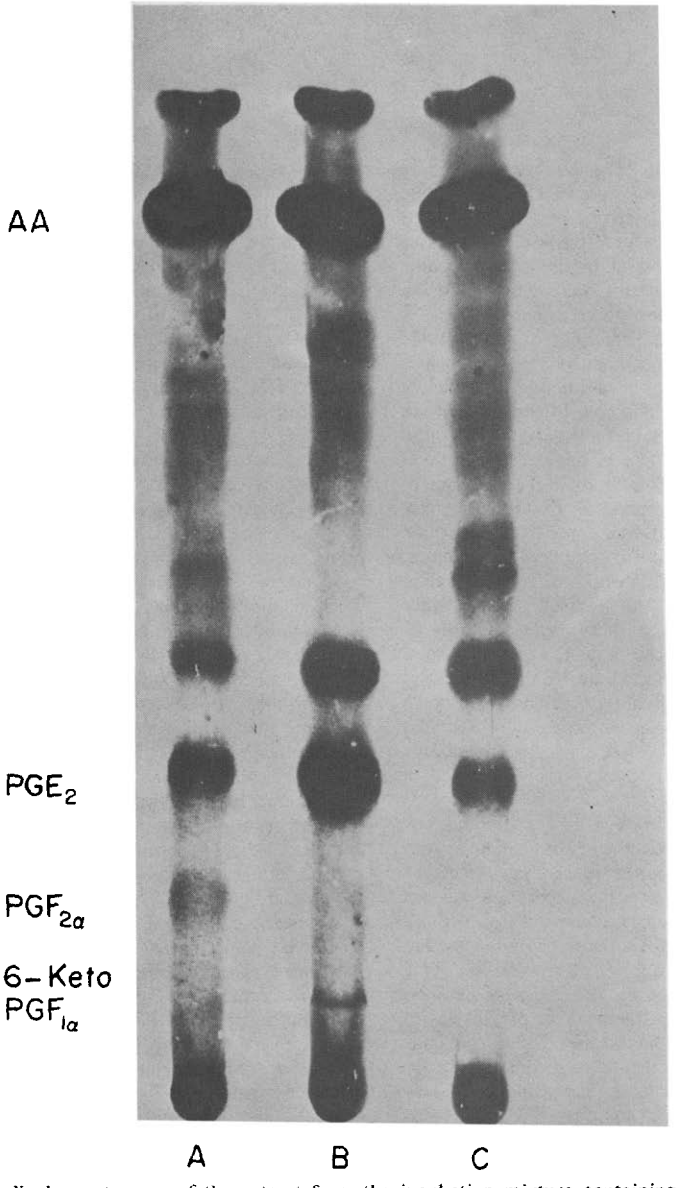

Fig. 3. Radiochromatogram of the extract from the incubation mixture containing ram seminal vesicle microsomes (RSVM) and 100 μM of sodium arachidonate + 1.64 nmoles (0.1 μCi) of [1-^{14}C] sodium arachidonate. Experimental conditions were the same as in Fig. 1. A. RSVM + arachidonic acid. B. RSVM + arachidonic acid + 1000 μM of chlorpromazine. C. RSVM + arachidonic acid + 100 μM of 6-hydroxydopamine.

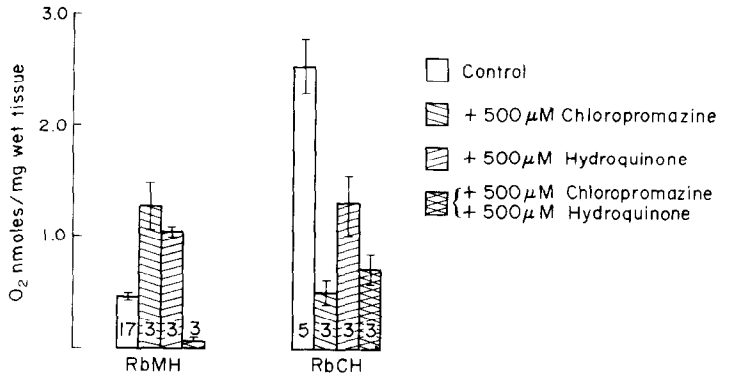

Fig. 4. The influence of chlorpromazine, hydroquinone and both compounds on the oxygen consumption in rabbit renal medulla (RbMH) and cortex (RbCH) homogenates. RbMH contained 100 mg of wet tissue/ml of 0.05 M phosphate buffer, pH 7. Oxygen consumption was read during 1 minute after 100 μM of sodium arachidonate was added. RbCH contained 20 mg of tissue/ml of the same buffer and oxygen consumption being read 5 min after sodium arachidonate was added.

$PGF_{1\alpha}$ were put on chromatographic plates. After developing of the plates spots of the standards were visualized in iodine vapours and compared with radioactive spots. Reagents were from the same sources as previously [10]. Additionally chlorpromazine from "Polfa" Poland and 6-hydroxydopamine from Sigma were used. The results were analysed using Student's t test and S.E. was shown everywhere.

The influence of chlorpromazine and 6-hydroxydopamine on oxygen consumption by ram seminal vesicle microsomes (RSVM) is shown in Fig. 1. Both compounds completely abolished oxygen consumption observed in control samples before addition of arachidonic acid. Chlorpromazine stimulated strongly arachidonic acid-induced oxygen consumption especially in the first minute after addition of the substrate. 6-Hydroxydopamine influenced oxygen consumption only slightly. The results presented in Fig. 1 were typical for the incubation mixture made from freshly prepared microsomes. During storage of lyophylized RSVM in − 20° the degree of stimulation by chlorpromazine decreased and 6-hydroxydopamine exerted a pronounced inhibitory effect. In Fig. 2 results of the experiments on the influence of tested compounds on the oxygen consumption by RSVM and rabbit kidney medulla homogenates (RbMH) are shown. Oxygen consumption by RbMH was taken as the measure of cyclo-oxygenase activity because it was stimulated by arachidonic acid and strongly suppressed (by 95%) by 100 μM of indomethacine.

To test the specifity of cyclo-oxygenase stimulation by chlorpromazine we compared the influence of it on the oxygen consumption by rabbit kidney cortex homogenates (RbCH). In this biological preparation oxygen consumption was not related to cyclo-oxygenase activity because it was not stimulated by arachidonic acid and not suppressed by indomethacine. This consumption was 2.51 ± 0.24 nmoles O_2 per mg wet tissue ($n = 15$) in control samples and 2.1 ± 0.64 nmoles O_2 per mg wet tissue ($n = 4$) in the presence of 100 μM of indomethacine (difference not significant). Chlorpromazine (1000 μM) significantly diminished oxygen consumption by RbCH. It was 0.42 ± 0.14 nmoles O_2 per mg wet tissue ($n = 3$) in the presence of chlorpromazine ($P < 0.001$; Fig. 4). 6-Hydroxydopamine did not change oxygen consumption by this biological preparation. It was 2.74 ± 0.27 nmoles O_2 per mg wet tissue ($n = 5$) in the presence of 100 μM of 6-hydroxydopamine (not significant).

The stimulation of arachidonic acid metabolism by chlorpromazine was confirmed using a radiochromatographic method as shown in Fig. 3.

The results of quantitative experiments were as follows: arachidonic acid conversion in control samples was 27.0 ± 1.1 per cent ($n = 3$). It was increased to 50 per cent in the presence of 1000 μM of chlorpromazine and decreased to 18 per cent in the presence of 6-hydroxydopamine (100 μM). Chlorpromazine stimulated 6-keto-$PGF_{1\alpha}$ generation by 70 per cent, PGE_2 generation by 570 per cent and diminished $PGF_{2\alpha}$ generation by 30 per cent. 6-Hydroxydopamine had no influence on 6-keto-$PGF_{1\alpha}$ and PGE_2 generation but inhibited $PGF_{2\alpha}$ generation by 40 per cent.

Hydroquinone abolished completely the effect of chlorpromazine on cyclo-oxygenase activity (Fig. 4). Both compounds stimulated oxygen consumption by RbMH when used separately. Treatment of the homogenate with both compounds together diminished oxygen consumption below control values. This effect was also specific for cyclo-oxygenase. In RbCH both compounds used separately of together diminished oxygen consumption.

The results of our experiments show that chlorpromazine is not an inhibitor but a stimulator of arachidonic acid metabolism. Previously observed inhibition [6–9] can be explained by the presence of cofactors in the incubation mixture or by very low substrate concentration (our unpublished results). We found that the stimulatory effect of chlorpromazine, as with that of paracetamol [11] and pyrazol–pyridine derivatives [10], is abolished in the presence of hydroquinone in the incubation mixture. All known free radical scavengers [1–4] stimulate cyclo-oxygenase. Chlorpromazine is not an exception. Free radical generator 6-hydroxydopamine has practically no influence on cyclo-oxygenase in spite of its strong auto-oxidand properties [5] and phenolic character which was supposed to be a typical feature of PG synthetase stimulators [2]. The inhibitory effect of free radical on cyclo-oxygenase has not been confirmed positively in the case of 6-hydroxydopamine.

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Diminished adenylate cyclase responses in frontal cortex and cerebral capillaries of spontaneously hypertensive rats

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A variety of neurohumoral agents, namely beta-adrenergic agonists, dopamine, histamine and prostaglandins, have been shown to stimulate the enzyme, adenylate cyclase, in the frontal cortex and cerebral neurons of laboratory rats [1]. Recently, capillary fractions from the rat cerebral cortex were found to contain a similar adenylate cyclase system sensitive to activation by beta-adrenergic agonists and dopamine [1–3]. In addition, the presence of adrenergic and dopaminergic nerve endings has been demonstrated in cerebral capillaries, and preliminary evidence suggests an involvement of norepinephrine-coupled adenosine cyclic 3′,5′-monophosphate (cyclic AMP) synthesis in the control of capillary permeability, particularly to water [4–9]. Under conditions of chronic hypertension, both blood–brain barrier lesions and cerebral edema development are known to occur, possibly as a result of increased cerebrovascular permeability [10–13]. The latter may indicate a defect in the capillary adenylate cyclase system. An extension of the latter hypothesis may be made to peripheral organs because activation of adenylate cyclase by neurohumoral agents has been shown to be reduced in the myocardium and blood vessels of hypertensive rats [14–17]. In addition, neuronal sites within the frontal cortex have been implicated in the pathogenesis of hypertension [18], and two popular antihypertensive drugs, propranolol (beta-adrenergic antagonist) and clonidine (alpha-adrenergic agonist), when given to spontaneously hypertensive rats (SHR), were found to alter the sensitivity of cortical adenylate cyclase to norepinephrine [19]. Against this background, the present study was designed to evaluate, in the frontal cortex and cerebral capillaries, whether the activation of adenylate cyclase by specified neurohumoral agonists differed between SHR and normotensive rats.

The animals, Wistar Kyoto (WKY) or SHR (obtained from Charles River Breeding Co., LA), were age matched per individual experimental condition (age range was 6.5 to 8 weeks), and the weight ranges were 135–175 g. The rats were decapitated, the brains were rapidly removed, and the cerebral cortices were dissected free and placed in cold (4°) 0.25 M sucrose (100 ml for the capillary isolation) or diglycine buffer (2 mM + 1 mM $MgSO_4$ + 0.2 mM EGTA,* pH 7.4, for the total frontal cortex). For the capillary isolation, the cortices (five per experiment) were freed of pia, chopped into 1 mm sections and filtered through successive pore sizes of nylon bolting cloth (333 and 110 μm, three times each). The suspension was centrifuged at 1500 *g* for 10 min, and the pellet was resuspended in 0.25 M sucrose, filtered (110 μm cloth), and recentrifuged. The pellet was again suspended in 0.25 M sucrose (40 ml), filtered (110 μm cloth) and layered onto a gradient consisting of 1.5, 1.3 and 1.0 M sucrose (5 ml each) and centrifuged at 20,000 rev/min (30 min) using a SW 25.1 rotor. The final pellet containing the capillary fraction, as monitored by phase microscopy [1, 8, 9, 20], was homogenized in cold diglycine buffer and, along with the frontal cortical homogenate, was assayed for adenylate cyclase activity (6 min at 37°) exactly as described previously [1, 2]. Enzyme activity was expressed as pmoles of cyclic AMP formed/mg of protein/min. Enzyme activity in the capillary fractions represented about $\frac{1}{3}$ of the total activity in the cortical homogenate. However, the total homogenate contains structures, e.g. myelin, which possess little enzyme activity. Moreover, other studies show that cellular purification yields progressively to higher adenylate cyclase activities [1]. Student's two-tailed *t*-test was used to compare data between the WKY and SHR, while Student's paired *t*-test was employed to evaluate enzyme stimulation over respective basal activity within a particular strain of rats.

Figure 1 compares the activation of adenylate cyclase in the frontal cortex of the WKY and SHR. Significant ($P < 0.05$) enzyme stimulation (when compared to respective basal activity) was produced in both animal strains by *l*-norepinephrine, dopamine, *d,l*-isoproterenol, histamine (all obtained from the Sigma Chem. Co., St. Louis, MO), salbutamol ($beta_2$-agonist, Allen & Hanbury's Ltd., England), and dobutamine ($beta_1$-agonist, gift from the Eli Lilly Co., Indianapolis, IN). In general, this enzyme activation was evident using agonist concentrations of 1–100 μM; however, in the SHR, dobutamine and salbutamol were not significantly effective at either 1 or 10 μM and histamine was likewise ineffective at 1 μM. In addition, there were significant differences in the activities of adenylate cyclase between the WKY and the SHR. Responses in the SHR were thus lower to norepinephrine (10 μM), dopamine (1–100 μM) and dobutamine (1–100 μM). In general, agonist-induced enzyme activation was lower throughout the SHR; however, the basal enzyme activities were not significantly different between the two strains.

In the cerebral capillary fraction from both strains, adenylate cyclase was elevated significantly by the adrenergic agonists alone (usually 1–100 μM). Histamine was not active, a finding reported previously [1, 2]. In the SHR, significant enzyme stimulation (over respective basal activity) was not evident at the lowest (1 μM) concentrations of norepinephrine, dobutamine or salbutamol. Reduced (significant) adenylate cyclase responses to norepinephrine (1–10 μM), dopamine (1–100 μM), isoproterenol (1–10 μM), salbutamol and dobutamine (both at 1–100 μM) were prominent in the SHR

* EGTA = ethyleneglycolbis(aminoethylether)tetraacetate.