prolyl hydroxylase to other processes involved in collagen biosynthesis such as synthesis, degradation and cellular secretion of collagen, certain tissues from animals treated for multiple days must be utilized.

March 23, 1979

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Metergoline antagonism of 5-hydroxytryptamine-induced activation of rat cerebral cortical (Na^+-K^+)ATPase

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It has been known for some time that biogenic amines (namely, noradrenaline, 5-hydroxytryptamine (5-HT), dopamine and histamine) produce a depressant effect on cerebral cortical and other central neurons (Krnjevic & Phillis 1963; Phillis 1970; Yarbrough et al 1974). The studies investigating the underlying mechanisms of biogenic amine action on neurons have suggested that amines may inhibit neurons by stimulating an electrogenic ion pump (Phillis 1976; Phillis et al 1978). Sastry & Phillis (1977) have shown that biogenic amine-induced depression of cerebral cortical neurons can be antagonized by (Na^+-K^+)-ATPase inhibitors and suggested that a major component of depressant action of monoamines may result from stimulation of an electrogenic (Na^+-K^+)-ATPase, an enzyme known to be associated with sodium pumping.

The biogenic amine-induced activation of (Na^+-K^+)-ATPase has been well documented (Schaefer et al 1972; Yoshimura 1973; Godfraind et al 1974; Lee & Phillis 1977; Phillis et al 1978). The mechanism by which noradrenaline exerts its effect on the (Na^+-K^+)-ATPase has also been examined. Noradrenaline stimulation o-(Na^+-K^+)-ATPase is antagonized by both α- and βt adrenoceptor blockers (Iwangoff et al 1974; Gilberf et al 1975; Wu & Phillis 1978) indicating that α- and β-adrenoceptors might be involved in the noradrenaline stimulation of (Na^+-K^+)-ATPase. The possibilities that 5-HT exerts its action on central neurons by a similar mechanism, namely, the stimulation of (Na^+-K^+)-ATPase, therefore warrant consideration. 5-HT activates brain (Na^+-K^+)-ATPase (Logan & O'Donovan 1976; Lee & Phillis 1977). However, the underlying mechanism(s) by which it does so are still poorly understood.

In the present investigation we have studied the stimulating effect of 5-HT and its structural analogues on the (Na^+-K^+)-ATPase of cerebral cortical homogenates, as well as the possibility of a 5-HT receptor involvement in the enzyme activation.

The cortex from male Sprague Dawley rats (200–300 g) was removed and homogenized in 50 vol of distilled H_2O (pH 7·5 buffered by Tris-HCl solution). Some of this homogenate (50 μl) was used for the incubation. The (Na^+-K^+)-ATPase activity was determined by subtracting Mg^{2+}-ATPase (ouabain insensitive) from total ATPase activity. The medium used for the estimation of total ATPase activity consisted of (mM) in final concentration: Tris, 115; $MgCl_2$, 5·0; KCl, 6·25; NaCl, 72·5; ATP, 2 pH 7·5. Mg^{2+}-ATPase activity was measured in the K^+-free medium which was composed of (mM) in final concentration: Tris, 172·5; $MgCl_2$, 5·0; NaCl, 14; ouabain, 1·0 and ATP, 2 pH 7·5. In all experiments, the homogenate was preincubated for 10 min at 37 °C in the presence of agonist and/or antagonist. The reaction was terminated 6 min after the addition of disodium adenosine triphosphate (ATP) by adding 1·0 ml ice-cold 12% trichloroacetic acid. The content of inorganic phosphate in the supernatant was measured by the method of Fiske & Subbarrow (1925). The reagents were made up freshly for every experiment. Metergoline was dissolved in small amounts of ethanol and subsequently diluted to a final concentration of 10^{-5} M containing 0·08 M ethanol.

Adenosine-5'-triphosphate(ATP) (Grade 1, synthesized by phosphorylation of adenosine), 5-hydroxytryptamine-oxalate, 5-hydroxyindolacetic acid and tryptamine were purchased from Sigma Chemical Co. (Mo., U.S.A.). Metergoline was a gift from Drs. Praga and Ghione of Farmitalia. Other chemicals were of analytical grade.

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In the present study, we have observed that $(Na^+–K^+)$-ATPase activity in rat cerebral cortical homogenate was significantly increased by 5-HT-oxalate (Fig. 1). This stimulatory effect is not due to removal of Ca^{2+} by oxalate because the addition of oxalate in concentrations ranging from 10^{-7}—10^{-3} M failed to alter $(Na^+–K^+)$-ATPase activity. Stimulation is specific to 5-HT, since tryptamine, a 5-HT analogue, was devoid of any activity on the enzyme. 5-HIAA is ineffective in enzyme stimulation at low concentrations, however, at 10^{-3} M, 5-HIAA stimulates $(Na^+–K^+)$-ATPase activity to 14% above its basal level ($P < 0.01$). This stimulation of $(Na^+–K^+)$-ATPase activity at high concentrations of 5-HIAA could reflect a non-specific action of the compound on $(Na^+–K^+)$-ATPase. Fifty percent of enzyme activation (E50) was found at a 5-HT concentration of 7×10^{-6} M, which is comparable to the E50 value for isoprenaline stimulation of the rat brain cortical $(Na^+–K^+)$ATPase, (Wu & Phillis 1979). 5-HT activates Mg^{2+}-ATPase (18%) only at high concentrations (10^{-3} M). Thus the stimulatory effect of 5-HT on the total ATPase system is almost entirely due to an increase in $(Na^+–K^+)$-ATPase activity.

Metergoline, [[(8β)-1,6-dimethylergolin-8-yl]-methyl]-carbamic acid phenylmethyl ester is a potent 5-HT receptor antagonist in various peripheral tissues (Beretta et al 1965, 1967) and in the central nervous system (Clineschmidt & Lotti 1974; Fuxe et al 1975; Sastry & Phillis, 1977). A study of the specificity of metergoline antagonism of 5-HT in the rat cerebral cortex has shown that metergoline antagonizes 5-HT-induced depressant responses, but not the responses generated by noradrenaline, histamine or adenosine monophosphate. The blockade of 5-HT evoked activation of $(Na^+–K^+)$-ATPase by the compound would, therefore, provide evidence for the involvement of a 5-HT receptor in the activation of $(Na^+–K^+)$-ATPase. As shown in Table 1, 23% of enzyme activation was elicited by 10^{-6} M of 5-HT-oxalate. This activation was completely abolished by 10^{-5} M metergoline. The receptor blockade was not reversed in the presence of equal concentrations of 5-HT, suggesting that metergoline has a higher affinity for the receptor than does 5-HT. As the metergoline was dissolved in ethanol, the media used for the experiments described in Table 1 contained a final concentration of 0·08 M ethanol. Since Israel et al (1965) have shown that high concentrations of ethanol can exert an inhibitory effect upon rat cerebral microsomal $(Na^+–K^+)$-ATPase, we have also investigated the effect of 0·08 M ethanol on $(Na^+–K^+)$-ATPase activity. At this concentration, ethanol had no inhibitory effect on rat cerebral cortical $(Na^+–K^+)$-ATPase (control 1·00 (0·12 s.d.) n = 11, ethanol 0·08 M 1·3 (0·07 s.d.) n = 6 μmol Pi mg^{-1} tissue h^{-1}. The antagonism shown in Table 1 must, therefore, be due to metergoline and not to an effect of the ethanol present in the medium. In conclusion, $(Na^+–K^+)$-ATPase of rat brain cortical homogenate is stimulated by 5-HT-oxalate. The stimulatory effect on the enzyme is specific to 5-HT since tryptamine and 5-HIAA are both inactive at 10^{-4} M concentrations. Activation of the enzyme may be mediated by a 5-HT receptor, because the stimulation of $(Na^+–K^+)$-ATPase activity evoked by 5-HT can be

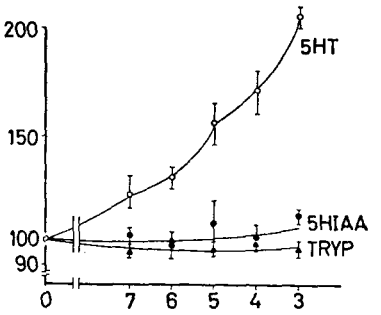

FIG. 1. Effect of 5-HT and its analogues on the stimulation of $(Na^+–K^+)$-ATPase of rat cerebral cortical homogenates. 1 mg of rat brain cortical tissue was preincubated with 5-HT or its analogues for 10 min. Inorganic phosphate was measured during the 6 min of incubation with substrate ATP immediately following the preincubation period. Results are presented as the mean with s.d. of four to six experiments. (–○–) represents $(Na^+–K^+)$-ATPase activity measured in the presence of 5-HT-oxalate; (–●–) indicates the enzyme activity in the presence of 5-HIAA and (–▲–) shows the enzyme activity in the presence of tryptamine. Ordinate: relative enzyme activity (% of control). Abscissa: −log [agonist concentration (M)].

Table 1. Antagonism of metergoline on the 5-hydroxytryptamine evoked stimulation of $(Na^+–K^+)$-ATPase in rat brain cortex

5-Hydroxytryptamine concentration (M)	Enzyme activity (percent of control) Absence of metergoline (10^{-5} M)*	Presence of metergoline (10^{-5} M)*
Control (0)	100	110 (6)
10^{-6}	123 (10)[2a]	100 (7·8)[1a]
10^{-5}	135 (6·8)[2b]	105 (5·3)[1b]

Each value is the mean with s.d. of the four experiments. 1 mg of rat brain cortical tissue was incubated with the media described in the text. The inorganic phosphate released during 6 minutes of incubation at 37 °C was measured by the method of Fiske & Subbarrow (1925).

1. The *t*-test was applied to compare the levels of enzyme activity measured in the presence or absence of metergoline.
2. The *t*-test was applied to compare the levels of enzyme activity in the presence of various concentrations of 5-hydroxytryptamine.

* 0·08 M ethanol was always present in the incubation medium.

a. $0.01 > P > 0.001$.

b. $0.001 > P$.

effectively blocked by metergoline. A receptor mediated reaction might be expected to show a saturation effect. However, in our experiments, 5-HT at 10^{-3} M does not have such an effect. It is possible that stimulation of (Na^+–K^+)-ATPase by 5-HT is mediated by more than one mechanism, as has been envisaged by us for the activation of (Na^+–K^+)-ATPase by noradrenaline (unpublished observations). Further studies on this receptor-mediated 5-HT stimulation of (Na^+–K^+)-ATPase should provide an insight into the mechanism by which 5-HT generates its depressant action on cerebral cortical neurons.

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Inhibition of [^{3}H]naloxone binding in homogenates of rat brain by eseroline, a drug, with analgesic activity, related to physostigmine

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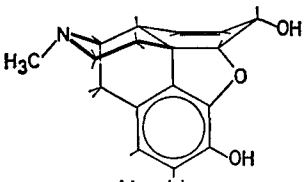
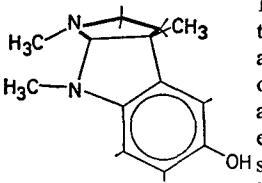
Eseroline, (3aS,8aR)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo [2,3-b] indol-5-ol, is a new analgesic drug derived from physostigmine by hydrolysis of *N*-methyl carbamyl group (Bartolini et al 1978). Eseroline, as free base, is unstable and readily oxidizes to the quinone derivative, rubreserine (Robinson 1965). Its



Morphine



Eseroline

salts, however, are more stable and can be stored as dry powders or in solution in the presence of an antioxidant, without noticeable loss of activity: in this study we have used the salicylate. Eseroline possesses a remarkable antinociceptive activity comparable in potency to that of morphine: the analgesic doses for eseroline salicylate range from 1 to 5 mg kg^{-1} s.c. in rats and mice depending on the test used (Bartolini et al submitted). In this respect eseroline resembles physostigmine, which also possesses antinociceptive activity in various tests (Flodmark & Wramner 1945; Hendershot & Forsaith 1959; Harris et al 1969; Pleuvry & Tobias 1971). Unlike the latter compound, however, eseroline is devoid of anticholinesterase activity (Ellis et al 1943, confirmed by our experiments on human blood serum) and its analgesic effect is not antagonized by atropine (Bartolini et al 1979). Since the antinociceptive actions of physostigmine are generally considered a consequence of its indirect cholinomimetic activity (Harris et al 1969; Ireson 1970), it appeared of interest to acquire more information on the mechanism of the analgesic action of eseroline.

In the present study we have examined the possibility of a direct interaction between eseroline and opiate receptor sites. To this end, we have evaluated the ability of eseroline in inhibiting stereospecific [^{3}H]-

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