oxidation was chromatographed both as the free diol and as its benzoyl ester. In no case did a spot appear with the R_f of a *cis*-cycloalkanediol.

The oxidation of benzocycloalkenes in mammalian liver microsomes has been shown to result in the formation of glycols. In the metabolism of 1,2-dihydronaphthalene[5] and of indene,[6] *trans*-diols were found; in the latter case, the *trans*-isomer was shown to be the preponderant or sole product, since no *cis*-diol could be demonstrated by methods which would have detected quite small quantities. In both of these hydrocarbons, the olefinic double bond is situated α, β to the benzene ring. The same is true of styrene, which is also oxidized to a glycol in liver microsomes.[4] The present experiments show that the simpler cycloalkenes, in which there is no aromatic ring, are also oxidized in these organelles, and that the products, as in the cases of the benzocycloalkenes, are predominantly or solely of the *trans*-configuration.

The epoxides of styrene, indene, and cyclohexene are hydrated in liver microsomes.[10] In the cases of the cyclic compounds, the diol products are of the *trans*-configuration, the same isomeric form as that obtained in the overall oxidation of the hydrocarbons. Styrene oxide[11] and octene oxide[12] have been shown to be intermediates in the conversion of the respective unsaturated hydrocarbons to glycols. Very recently, it has been demonstrated that the oxidation of cyclohexene to its *trans*-diol also involves the intermediate formation of an epoxide.[13] The same pathway is probably involved in the oxidations of the other cycloalkenes reported here.

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Modification by psychotropic drugs of the cyclic adenosine monophosphate response to norepinephrine in rat brain*

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THE CATECHOLAMINES are thought to play an important role in brain function, and have been implicated in the mode of action of a number of psychotropic drugs.[1] Many of the peripheral actions of the catecholamines are known to be mediated by changes in the intracellular level of adenosine 3′,5′-monophosphate (cyclic AMP),[2] and there is now evidence to suggest that some of their central

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effects may be similarly mediated. Brain tissue contains the highest activity of adenyl cyclase of any of the mammalian tissues which have been studied.[3] Subcellular distribution studies[4] suggest that a substantial fraction of this activity may be located in synaptic membranes, and studies with pineal gland homogenates[5] suggest that at least part of this activity may be located postsynaptically. Although homogenates of most brain areas respond poorly, if at all, to hormones,[6–9] adenyl cyclase in washed cerebellar particles can be stimulated by catecholamines.[6] The catecholamines have been shown to increase the level of cyclic AMP in slices or chopped tissue preparations of the brains of all species examined,[10–13] and the response to norepinephrine is increased after chronic administration of reserpine.[12] At least one central adrenergic response, the inhibition of Purkinje cell firing in the rat cerebellum, can be mimicked by the application of exogenous cyclic AMP.[14]

Rall *et al.*[10,15,16] have shown that the stimulation of brain cyclic AMP formation by several stimuli, including norepinephrine, can be inhibited by chlorpromazine. We have now studied the effect of chlorpromazine and several other agents on the ability of norepinephrine to increase the level of cyclic AMP in slices of the hypothalamus and brain stem of the rat. The results of these studies are reported herein.

Young adult male Sprague–Dawley rats (200–250 g) were used. The methods employed for the preparation and incubation of tissue samples were essentially those of Kakiuchi and Rall.[10] Briefly, the animals were decapitated in a cold room and the brains rapidly removed and placed in ice-cold pH 7·4 buffer. The hypothalamic and brain stem areas were divided into right and left halves, one side serving as a control for the other side. The brain tissue was chopped using a McIlwain tissue chopper (0·275 mm), and the tissue samples were added to beakers containing 30 ml of pH 7·4 buffer, stirred, the medium was removed and 30 ml of fresh medium was added. The beakers were then placed in a Dubnoff metabolic shaker and preincubated at 37°, with 95% O_2–5% CO_2 bubbled into individual samples. Thirty min later, the medium was removed and replaced with 20 ml of fresh medium. At this time, either drugs or solvents (control) were added to the samples. After an additional 14 min of incubation, norepinephrine (5×10^{-5} M) or the control solution was added. After 6 min, the samples were homogenized in a Waring blender that contained 2 ml of 1 N HCl and a known amount of tritiated cyclic AMP. An aliquot of the homogenate was used for the determination of

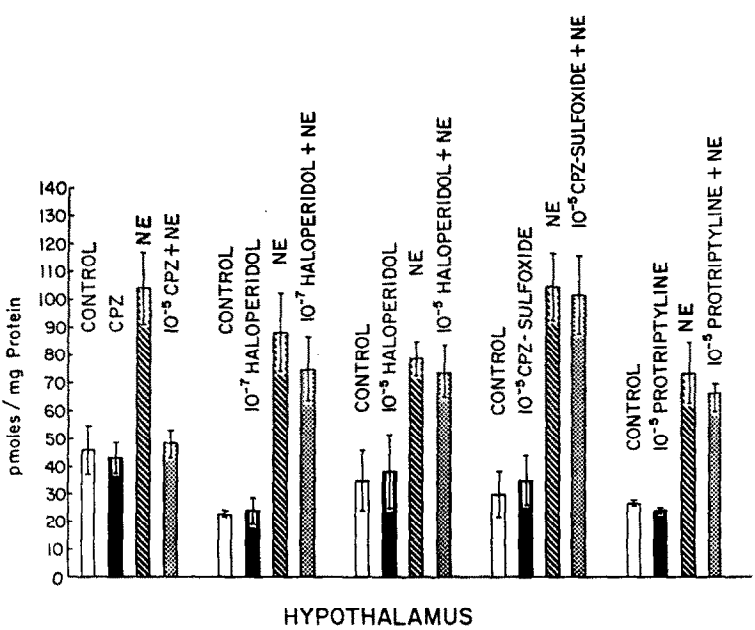

FIG. 1. Modification by psychotropic drugs of the cyclic AMP response elicited by norepinephrine in chopped tissue of the rat hypothalamus. The results are expressed as picomoles per milligram of protein. Vertical bars represent the standard error of the mean of at least three paired assays. CPZ, 10^{-5} M chlorpromazine; NE, 5×10^{-5} M *dl*-norepinephrine; CPZS, 10^{-5} M chlorpromazine sulfoxide

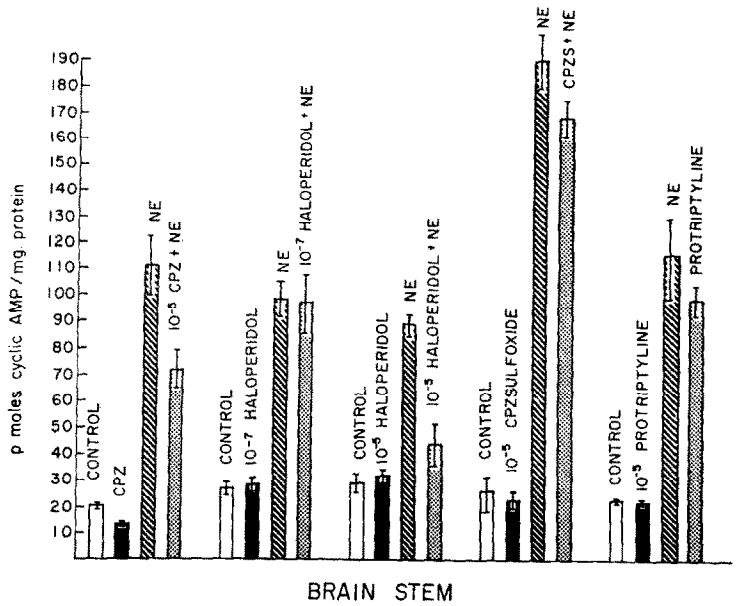

FIG. 2. Modification by psychotropic drugs of the cyclic AMP response elicited by norepinephrine in chopped tissue of the rat brain stem. For details, see Fig. 1.

proteins according to Lowry *et al.*[17] The remainder was centrifuged at 2000 *g* and the cyclic AMP was isolated and determined by a modification of the method of Butcher *et al.*[18]

Norepinephrine (5×10^{-5} M) elicited a 2- to 5-fold increase in cyclic AMP in chopped tissue preparations from the hypothalamus and the brain stem. Chlorpromazine (10^{-5} M) partially blocked the increase in cyclic AMP evoked by norepinephrine in both the hypothalamus and brain stem (Figs. 1 and 2). These findings are in agreement with those of Kakiuchi and Rall,[10,11] who found that chlorpromazine antagonized the norepinephrine-induced increase in cyclic AMP in slices of rabbit cortex and cerebellum. Haloperidol (10^{-5} M) also inhibited the norepinephrine-induced increase in cyclic AMP in the brain stem, but did not affect the response elicited by norepinephrine in the hypothalamus. These findings are of interest since haloperidol, a butyrophenone derivative, has pharmacological properties which closely resemble those of the phenothiazines. As a means of examining the specificity of the action of chlorpromazine on the norepinephrine-induced elevation of cyclic AMP, the effects of chlorpromazine sulfoxide, a pharmacologically inactive metabolite of chlorpromazine, and of protriptyline, a potent tricyclic antidepressant, were investigated. Neither chlorpromazine sulfoxide nor protriptyline (10^{-5} M) blocked the norepinephrine-induced increase in cyclic AMP in the hypothalamus or the brain stem or changed the basal levels of the cyclic nucleotide (Figs. 1 and 2).

It is noteworthy that the addition to the incubation medium of the monoamine oxidase (MAO) inhibitor, pargyline (10^{-5} M), caused an elevation of the levels of cyclic AMP (Fig. 3). However, the MAO inhibitor did not modify the cyclic AMP response evoked by norepinephrine. Similar results were obtained when pargyline was injected into rats 18 hr prior to sacrifice (75 mg/kg, i.p.).

Chlorpromazine and haloperidol, drugs with entirely different chemical structures but with an almost identical pharmacological profile, including central adrenergic blocking properties, inhibited the norepinephrine-induced increase in the level of cyclic AMP in chopped tissue preparations of the rat brain stem (haloperidol) or of both rat brain stem and hypothalamus (chlorpromazine). It is noteworthy that chlorpromazine sulfoxide, a pharmacologically inactive chlorpromazine metabolite, and the structurally related tricyclic antidepressant, protriptyline, did not exert similar blocking properties.

The mechanism by which chlorpromazine and haloperidol might act to inhibit this response is quite obscure at present. Chlorpromazine is also capable of inhibiting the accumulation of cyclic AMP in rabbit cerebellar slices in response to histamine[10] as well as in guinea pig cortex in response to electrical stimulation.[15] It is possible that the latter effect is mediated by adenosine.[16] Chlor-

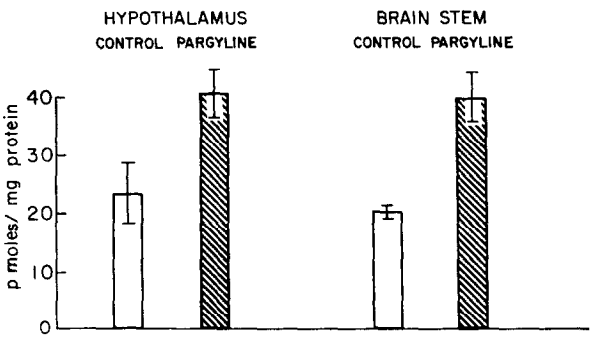

FIG. 3. Effect of pargyline on the basal level of cyclic AMP in chopped tissue of the rat hypothalamus and brain stem. Vertical bars represent the standard error of the mean of four paired assays.

promazine has been shown to inhibit both adenyl cyclase[19] and phosphodiesterase[20] in broken cell preparations from other tissues, but whether the specificity in these systems is as we have found it in the rat brain remains to be seen.

In any event, the specificity observed in the present experiments supports the view that chlorpromazine and chlorpromazine-like drugs could owe at least part of their pharmacological activity to their ability to inhibit the accumulation of cyclic AMP in certain cells within the central nervous system. It is obvious that a great deal of additional research will be required in order to establish this hypothesis.

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