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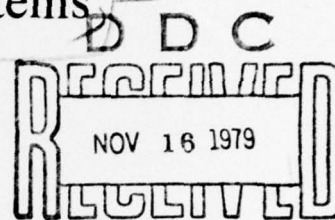
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6 Effects of Acute and Chronic Administration of Ethanol on Cyclic Nucleotides and Related Systems 8

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A. INTRODUCTION

Over the past 10-20 years the possible role of cyclic nucleotides in biological function has received considerable attention. It is now believed that cyclic nucleotides act as a "second messenger" in the actions of a variety of hormones. Since a physiological response could result from the disruption of any one of a chain of events, the possible role of cyclic nucleotides in the actions of ethanol both after acute and chronic administration have been explored.<sup>(1)</sup> It will be the purpose of this review to collate and analyze the existing data on ethanol-cyclic-nucleotide interactions and to determine if these effects could be responsible for some of the actions of ethanol.

In order to gain perspective it will be necessary to summarize the present theories on the role of cyclic nucleotides in biological function and how they accomplish their actions. Many excellent reviews<sup>(2-4)</sup> have been written exhaustively detailing what has been reported on cyclic nucleotides. Hence, our purpose here will be only to present basic concepts in order to help understand the potential relevance of the actions of ethanol on this system.

B. ETHANOL AND CYCLIC NUCLEOTIDES IN THE NERVOUS SYSTEM

1. General Aspects of Cyclic Nucleotide Function

Evidence to date supports a role of cyclic nucleotides in synaptic transmission. Cyclic nucleotides have been implicated in the actions of a number of neurotransmitters, including norepinephrine, dopamine, serotonin, acetylcho-

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line, GABA, and glutamate. Adenosine-3',5'-cyclic monophosphate (cyclic AMP) appears to be related to the effects of norepinephrine,<sup>(5-7)</sup> dopamine,<sup>(8,9)</sup> and serotonin,<sup>(10)</sup> while guanosine-3',5'-cyclic monophosphate (cyclic GMP) is related to the effects of acetylcholine,<sup>(11-14)</sup> GABA,<sup>(15)</sup> and glutamate.<sup>(16)</sup>

The current belief concerning the possible role of cyclic nucleotides in synaptic transmission involves the initial stimulation of a receptor by the neurotransmitter substance. In the case of biogenic amines, interaction with their receptors involves adenylate cyclase which converts ATP to cyclic AMP,<sup>(17)</sup> while acetylcholine, GABA, and glutamate act through guanylate cyclase, which converts GTP to cyclic GMP.<sup>(18)</sup> When these enzymes are stimulated, an accumulation of the corresponding cyclic nucleotide occurs. This elevated cyclic nucleotide level increases the activity of cyclic-nucleotide-dependent protein kinases, which appear to be separate entities, one specific for cyclic AMP and one for cyclic GMP.<sup>(19)</sup> These protein kinases catalyze the phosphorylation of proteins located in synaptosomal membranes,<sup>(20,21)</sup> and this process is believed to alter the permeability of the membrane to ions, which in turn changes the resting membrane potential.

The most detailed account of the interaction of neurotransmitters and cyclic nucleotides and the concomitant changes in the membrane potential has been with the superior cervical ganglion. Evidence accumulated here has been easier to interpret because of its relatively simple structural organization when compared to the brain. It now appears that dopamine is released from interneurons in the ganglion and stimulates the formation of cyclic AMP postsynaptically.<sup>(8,22,23)</sup> These events coincide with the development of the slow inhibitory postsynaptic potential.<sup>(24)</sup> On the other hand, acetylcholine released from preganglionic fibers stimulates the formation of cyclic GMP postsynaptically<sup>(23)</sup> and may be related to the slow excitatory postsynaptic potential.<sup>(25)</sup> This effect seems to be mediated through muscarinic receptors, since it can be blocked by muscarinic antagonists but not by nicotinic antagonists.<sup>(26)</sup>

Most of the research on the effect of ethanol on cyclic nucleotides has been on cyclic AMP and related systems in brain tissue. Measurements of brain cyclic AMP are very tricky especially in the cerebellum because of a rapid postmortem accumulation of this nucleotide.<sup>(27)</sup> Consequently, rapid inactivation of brain enzymes is very important. To accomplish this, high-intensity microwave irradiation has been used. Initially, a conventional microwave oven was tried,<sup>(27)</sup> but it required 20 sec to inactivate adenylate cyclase and phosphodiesterase. More recently a microwave oven was developed which focuses the beam on the head of an animal.<sup>(28)</sup> With this modification the enzymes are inactivated in 2 sec.

## 2. Effects of Ethanol on Cyclic AMP

### a. Acute Treatment

In an early study Volicer and Gold<sup>(29)</sup> reported, using conventional microwave irradiation for 45 sec, that a single 1- to 6-g-ethanol/kg dose, given orally,

depressed cyclic AMP levels up to 60% in whole brain of rats in a dose-dependent manner. This response was predominantly in the cerebellum. Orenberg *et al.*<sup>(30)</sup> examined cyclic AMP levels in several areas of the mouse brain after 9 sec of conventional microwave irradiation. In the cerebral cortex, cyclic AMP levels were depressed up to 65% 1 hr after treatment by 0.4- to 3.2-g-ethanol/kg doses, given intraperitoneally. However, under similar conditions, subcortical and cerebellar levels were significantly elevated by 60 and 170%, respectively, 10 min after treatment. When mice were killed by immersion in liquid nitrogen, ethanol had no effect on cyclic AMP levels in the cerebral hemispheres.<sup>(31)</sup> Recently, using a 9-sec exposure of focused microwave irradiation, Volicer and Hurter<sup>(32)</sup> found a dose-dependent reduction in cyclic AMP levels after 1-6 g ethanol/kg, given orally, in the cerebral cortex, cerebellum, and brain stem of the rat 1 hr after treatment. On the other hand, Redos *et al.*<sup>(33)</sup> were unable to demonstrate any alteration in cyclic AMP levels in any area of the brain 2 hr after treatment with a 6-g-ethanol/kg oral dose and using 3.5 sec of focused microwave irradiation.

The preceding discussion illustrates the uncertainty of the effect of single doses of ethanol on cyclic AMP levels in the brain. The source of these ambiguities is not clear but may be related to methodological considerations. A summary of some of the data can be found in Table I. As pointed out earlier, rapid inactivation of the synthetic and degradative enzymes for cyclic AMP is very important to minimize its postmortem accumulation in the brain and to reflect more closely the actual levels *in vivo*. In the studies to date, exposure durations vary from 3.5 to 9.0 sec for focused microwave irradiation and 9 to 45 sec using conventional microwave ovens. Although the enzymes can be inactivated fairly quickly, it is unclear whether the existing technology does it fast enough, especially in the cerebellum, where postmortem accumulation is most pronounced.<sup>(27)</sup> Also, ethanol inhibits this accumulation in rats<sup>(29)</sup> and could induce artifacts by making it appear that ethanol-depleted cyclic AMP, when in

Table I. Effect of a Single Dose of Ethanol on Cyclic AMP Levels in Brain

|                 | Dose         | Time after treatment | Mode of killing <sup>a</sup> | Cyclic AMP       |         | Reference |
|-----------------|--------------|----------------------|------------------------------|------------------|---------|-----------|
|                 |              |                      |                              | Control          | Treated |           |
| Cerebellum      | 4 g/kg, po   | 3 hr                 | CMI                          | 2.2 <sup>b</sup> | 1.5     | 29        |
|                 | 4 g/kg, po   | 1 hr                 | FMI                          | 1.4 <sup>b</sup> | 0.7     | 32        |
|                 | 6 g/kg, po   | 2 hr                 | FMI                          | 3.4 <sup>c</sup> | 3.2     | 33        |
|                 | 3.2 g/kg, ip | 10 min               | CMI                          | 0.6 <sup>b</sup> | 1.5     | 30        |
| Cerebral cortex | 4 g/kg, po   | 3 hr                 | CMI                          | 8.0 <sup>b</sup> | 7.0     | 29        |
|                 | 4 g/kg, po   | 1 hr                 | FMI                          | 1.4 <sup>b</sup> | 0.9     | 32        |
|                 | 6 g/kg, po   | 2 hr                 | FMI                          | 7.2 <sup>c</sup> | 7.2     | 33        |
|                 | 3.2 g/kg, ip | 10 min               | CMI                          | 1.4 <sup>b</sup> | 1.0     | 30        |
|                 | 4 g/kg, ip   | 1.5 hr               | ILN                          | 0.7 <sup>b</sup> | 0.7     | 31        |

<sup>a</sup>CMI, conventional microwave irradiation; FMI, focused microwave irradiation; ILN, immersion in liquid nitrogen.

<sup>b</sup>Values expressed as pmole/mg tissue.

<sup>c</sup>Values expressed as pmole/mg protein.



fact it might only have blocked the postmortem accumulation that could take place within the first few seconds after death. In further support of this possibility is the report of Jones and Stavinoha<sup>(34)</sup> that a significant elevation of cyclic AMP can occur in the brain under these circumstances. They compared the cyclic AMP levels in various parts of the brain of mice exposed either to 4 sec of 1.5 kW or 0.3 sec of 6 kW of focused microwave radiation. In all cases the cyclic AMP levels were higher with the lower intensity, longer duration irradiation. In the cerebellum, for example, the values increased by 100%.

Most of the studies using microwave irradiation expose the brain longer than is necessary to inactivate enzymes. The potential problems of overexposure have not been explored. For example, does prolonged high heat decompose cyclic AMP? Does the progressive cellular damage induced by continued exposure alter the distribution of cyclic AMP in a manner that might ultimately affect its quantitation? Or does interaction of ethanol and other drugs with the brain alter the dynamics of inactivation?

Different responses in cyclic AMP levels have been obtained in different areas of the brain after ethanol treatment and are a function of the dose and time after exposure. Most studies do not report blood ethanol concentrations, making any meaningful comparisons between studies difficult.

In summary, in order to determine what single doses of ethanol do to cyclic AMP levels in the brain, it is necessary to employ methods of killing animals that are well understood and standardized. Experiments need to be carried out carefully providing complete dose-response and time-course relationships accompanied by blood ethanol concentrations. Finally, the possibility of species differences should not be ignored.

#### **b. Chronic Treatment**

Chronic treatment with ethanol has produced results on cyclic AMP levels and related systems that are less controversial. If animals are treated for at least 8 days, cerebral cyclic AMP is elevated 70%.<sup>(31,32)</sup> However, after only 4 days of treatment, no increases in cyclic AMP are observed.<sup>(33)</sup> These increases in cyclic AMP appear to be related to an elevation in adenylate cyclase activity. Kuriyama and Israel<sup>(31)</sup> found that at a time when cyclic AMP levels were elevated, there was also an increase in adenylate cyclase activity. Neither response was observed after a single dose of ethanol. Also, phosphodiesterase activity was unaffected after either treatment.<sup>(31)</sup>

More interesting and possibly more functionally important findings are the studies determining the sensitivity of the cyclic AMP system to neurotransmitters. Present evidence is quite compelling that chronic administration of ethanol alters the sensitivity of cyclic-AMP-generating systems to norepinephrine. An early study demonstrated that treatment with ethanol for 14 days decreased the sensitivity of this system to norepinephrine.<sup>(35)</sup> French and co-workers have subsequently reported some very interesting results on changes of sensitivity of

cyclic AMP accumulation to neurotransmitters in rats after 16 weeks of ethanol treatment. The results observed depended on the time after withdrawal the measurements were made. In cortical slices obtained from animals withdrawn for 2 hr, the sensitivity of the cyclic-AMP-generating system to norepinephrine was depressed, with the dose-response curve shifting 4.3-fold to the right.<sup>(36)</sup> This development of noradrenergic subsensitivity could be explained by hyperactivity of noradrenergic nerve terminals.<sup>(37,38)</sup> However, it was not established whether a single dose of ethanol might produce the same result on cyclic AMP accumulation. Three days after withdrawal, a time corresponding to the development of delirium tremens,<sup>(39)</sup> the opposite response was observed. The sensitivity of the cyclic-AMP-generating system to norepinephrine increased 2.4-fold.<sup>(40,41)</sup> In addition, the sensitivity to histamine and 5-HT was also enhanced.<sup>(42)</sup> The response to 5-HT might result from a chronic depression of serotonergic function.<sup>(43)</sup>

The significance of alterations in the sensitivity of the cyclic-AMP-generating system to specific aspects of alcoholism has not been demonstrated. At the least they reflect changes induced by long-term consumption of ethanol. It is not clear whether they are related to the development of physical dependence, which was minimal with the methods used,<sup>(44)</sup> or whether they are expressions of some nonspecific neural toxicity. The whole question of adaptive mechanisms has considerable significance for explaining tolerance and physical dependence. If ethanol treatment induces alterations in neurotransmitter function (see Chapter 11) chronic changes can lead to compensatory changes in other parameters in an attempt to maintain homeostasis. The role of cyclic nucleotides in adaptive processes has received considerable attention and has been concisely reviewed recently by Dismukes and Daly.<sup>(2)</sup> Because of the potential importance of these findings, it is imperative that the studies of French and co-workers be repeated using an animal model that takes only a few days for inducing physical dependence. In this way, nonspecific neural toxicity can be discounted, and the relationship between neurotransmitter sensitivity and overt, spontaneous withdrawal signs will be a more convincing possibility.

Adaptive changes can take place not only in the sensitivity of receptors to neurotransmitters, but possibly also with alterations in the activity of the receptor for cyclic AMP, the cyclic-AMP-dependent protein kinase. As mentioned earlier, this enzyme catalyzes the phosphorylation of protein in synaptosomal membranes. Recently Kuriyama *et al.*<sup>(45)</sup> reported a study in which they measured cyclic-AMP-dependent protein kinase activity in mouse cerebral cortex after acute and chronic administration of ethanol. They found that after treatment for 2 weeks, synaptosomal enzymatic activity was elevated fourfold but returned to control levels 7 days after withdrawal. No alteration in protein kinase activity was observed after a single dose of ethanol. One problem with this work is that the conditions under which the animals were killed after chronic ethanol treatment were not defined, and the appearance of possible withdrawal signs was not reported. Thus, it is difficult to determine whether this alteration is related to the development of physical dependence. It appears, however, that the elevation

in cyclic-AMP-dependent protein kinase activity might be a consequence of the increased activity of adenylate cyclase and cyclic AMP levels<sup>(31)</sup> and not to an adaptive change in the sensitivity of protein kinase to cyclic AMP analogous to norepinephrine-induced cyclic AMP accumulation.

### 3. Effects of Ethanol on Cyclic GMP

The possible role of cyclic GMP in the actions of ethanol has been recently reported. Redos *et al.*<sup>(46)</sup> found that ethanol depletes cerebellar cyclic GMP in the rat in a dose-dependent manner. The maximum effect was observed 1 hr after oral administration of 6 g ethanol/kg, which resulted in a loss of 95% of the cyclic GMP. In addition, the degree of depletion was directly proportional to the blood ethanol concentration with control values obtained when ethanol had been eliminated. Similar results were found by Volicer and Hurter.<sup>(32)</sup>

Studies of the effects of ethanol on cyclic GMP have been expanded to include measurements in other areas of the brain and after both acute and chronic administration. Sample results can be found in Table II. Single doses of ethanol were found to deplete cyclic GMP in the cerebral cortex, caudate nucleus and thalamus as well as in the cerebellum.<sup>(32,47)</sup> In chronically treated rats rendered ethanol dependent, similar results were observed if the animals were still intoxicated. However, in the cerebellum and the brain stem, tolerance develops to the effect of ethanol on cyclic GMP levels<sup>(47)</sup> corresponding to the development of behavioral tolerance under similar experimental conditions.<sup>(48)</sup> When acutely and chronically treated animals are compared at similar blood ethanol concentrations, cyclic GMP levels were significantly higher in the chronically treated animals. If cyclic GMP levels are depressed for a long period, one might expect to see an overshoot after the elimination of ethanol. This was not observed after 4 days of treatment. Cyclic GMP levels in all areas of the brain studied were at control levels during the ethanol withdrawal syndrome.<sup>(47)</sup> When treatment was extended to 8 days, a significant elevation of cerebellar cyclic GMP was found.<sup>(32)</sup> However, since withdrawal signs develop with both treatment regimens, elevation of cerebellar cyclic GMP by itself does not appear to be a prerequisite for the expression of an ethanol withdrawal syndrome.

Table II. Effect of Ethanol Treatment on Cyclic GMP Levels in Brain<sup>a</sup>

|                 | Control          | Acute <sup>b</sup> | Chronic intoxicated | Chronic withdrawal | References |
|-----------------|------------------|--------------------|---------------------|--------------------|------------|
| Cerebellum      | 4.0 <sup>b</sup> | 0.2                | 1.2                 | 3.3                | 46, 47     |
|                 | 0.6 <sup>c</sup> | 0.3                | 0.3                 | 1.5                | 32         |
| Cerebral cortex | 0.5 <sup>b</sup> | 0.1                | 0.2                 | 0.4                | 46, 47     |
|                 | 0.1 <sup>c</sup> | 0.05               | 0.05                | 0.1                | 32         |

<sup>a</sup>Cyclic GMP levels were determined 1 hr after a single 6-g ethanol/kg dose given orally.

<sup>b</sup>Values expressed as pmole/mg protein.

<sup>c</sup>Values expressed as pmole/mg tissue.



The mechanism by which ethanol depletes cyclic GMP is unknown. An attempt to show a direct effect of ethanol in concentrations up to 200 mM on guanylate cyclase or cyclic GMP-phosphodiesterase activity *in vitro* was not successful.<sup>(47)</sup> However, the assays were performed under optimal enzymatic conditions which do not necessarily reflect what occurs *in vivo*. For example, calcium may regulate the activity of guanylate cyclase, and changes in the actions of calcium might depress the synthesis of cyclic GMP.<sup>(49-51)</sup> Ethanol has been shown to induce several alterations in calcium that might restrict the intracellular concentration of calcium and suppress guanylate cyclase activity. Ethanol can block the inward calcium currents in *Aplysia* neurons,<sup>(52)</sup> enhance calcium binding to erythrocyte ghost membranes,<sup>(53)</sup> and reduce brain calcium.<sup>(54)</sup> Although all these studies may not be relevant to an effect on guanylate cyclase in brain, they do indicate a need for more direct experiments into the possible role of calcium in the actions of ethanol.

Alterations in neurotransmitter activity might be involved in the ethanol-induced depletion of cyclic GMP. As indicated earlier, cyclic GMP appears to be related to the actions of acetylcholine, GABA, and glutamate. Ethanol has been demonstrated to inhibit cortical and reticular acetylcholine release *in vivo*<sup>(55)</sup> and depresses cerebellar glutamate levels.<sup>(56)</sup> Whether these changes could explain, at least in part, the depletion of cyclic GMP has yet to be determined.

Alterations in systems involving cyclic GMP could be involved in the expression of an ethanol withdrawal syndrome. Collier *et al.*<sup>(57)</sup> have studied the effect of agents which have actions related to cyclic nucleotides on ethanol withdrawal head twitches in mice. They found that dibutyryl cyclic GMP and GTP, compounds which elevate brain cyclic GMP, increase the incidence of head twitches over twofold, while dibutyryl cyclic AMP, ATP, and prostaglandins, compounds which elevate brain cyclic AMP, antagonize head twitches. These results support the view that an alteration in the balance of cyclic GMP and cyclic AMP in favor of the former might play a role in the expression of the withdrawal syndrome. Further, there is a parallel between harmaline- and withdrawal-induced tremors. Harmaline affects the cerebellum by stimulating the excitatory climbing fibers that synapse on the Purkinje cells<sup>(58)</sup> in a manner resembling activation of climbing fibers.<sup>(59)</sup> The tremor induced by harmaline is accompanied by an elevation in cerebellar cyclic GMP and can be blocked by prior administration of benzodiazepines.<sup>(60)</sup> These drugs have been used to treat the ethanol withdrawal syndrome in man.<sup>(61)</sup> Consequently, future research is warranted to try to find a possible role of cyclic GMP or related systems in ethanol dependence, possibly through alterations resulting from chronic cyclic GMP depletion.

### C. ETHANOL AND CYCLIC NUCLEOTIDES IN THE STOMACH

Ethanol is known to stimulate gastric acid secretion in a number of species, including man.<sup>(62)</sup> The response obtained is similar to that elicited by histamine,<sup>(63)</sup> but subsequent work ruled out a role of histamine in the effect of



ethanol.<sup>(64,65)</sup> Cyclic AMP has been implicated as a mediator in acid secretion,<sup>(66,67)</sup> and in addition the gastric mucosa contains a cyclic-AMP-dependent protein kinase which may be involved in translocation of ions.<sup>(68)</sup> Therefore, it is possible that changes in cyclic AMP metabolism may be related to the ability of ethanol to alter acid secretion.

The possible relationship between the effect of ethanol on gastric acid secretion and cyclic AMP metabolism has been explored in several species, including man. In the rat, local application of ethanol on the gastric mucosa in concentrations of 1–10% reduced acid secretion in a concentration-dependent manner from 30 to 100% and decreased cyclic AMP levels up to 50%.<sup>(69)</sup> In the dog, ethanol has a biphasic effect on acid secretion. In concentrations below 20% ethanol stimulates secretion, while at concentrations above 20% it depresses secretion.<sup>(70)</sup> Ethanol also exerts the same biphasic effect on mucosal cyclic AMP levels.<sup>(71)</sup> In man, ethanol concentrations that stimulate acid secretion are accompanied by increases in mucosal cyclic AMP levels.<sup>(72)</sup> It would appear, then, that alterations in gastric acid secretion as affected by ethanol are directly correlated with changes in cyclic AMP content.

In determining the mechanism by which ethanol alters cyclic AMP levels, a first step is to study the effect of ethanol on mucosal adenylate cyclase and phosphodiesterase activities. In the rat, ethanol inhibits both enzymes in a concentration-dependent fashion but with different sensitivities.<sup>(69,73)</sup> For example, at about 10% ethanol, phosphodiesterase activity is reduced by 50%, but adenylate cyclase is nearly completely blocked. In the dog and man, ethanol stimulates adenylate cyclase activity at low concentrations<sup>(71,72)</sup> but is ineffective at high concentrations when cyclic AMP levels and acid secretion are reduced.<sup>(71)</sup> As with the rat, phosphodiesterase in the dog is inhibited. These findings suggest that ethanol may elevate acid secretion by stimulating adenylate cyclase, but they do not explain how ethanol can depress secretion and cyclic AMP levels.

As pointed out earlier, assays *in vitro* are not always reliable in reflecting the true status in the living animal. Other factors often contribute to the rate of synthesis and degradation of a compound. Cyclic AMP falls into this category. It is well known that cyclic AMP levels in a variety of tissues are affected by a number of hormones, neurotransmitters, and ions. Ethanol may induce alterations in one of these other factors.

One factor that affects the content of cyclic AMP is the availability of its precursor ATP if adenylate cyclase is not saturated. Puurunen *et al.*<sup>(74)</sup> perfused the stomach with 10% ethanol and measured acid secretion and cyclic AMP and ATP levels in the whole gastric mucosa and in the superficial mucosa, where most of the acid-secreting parietal cells are located. After 40 min, all three parameters were significantly reduced in the superficial mucosa but not in the whole mucosa. When perfusion was discontinued, acid secretion and cyclic AMP and ATP returned to control levels in about 60 min. Since no additional cyclic AMP was detected in the gastric perfusate, the reduced cyclic AMP in the superficial mucosa cannot be explained by leakage of cyclic AMP from the mucosal cells. A reduction of mucosal ATP levels has also been observed in the dog after ethanol perfusion.<sup>(75)</sup>

Prostaglandins interact with cyclic AMP metabolism<sup>(76,77)</sup> and inhibit gastric acid secretion.<sup>(78,79)</sup> In an attempt to determine if prostaglandins might play a role in ethanol's depressant effect on acid secretion in the rat, Karppanen and Puurunen<sup>(80)</sup> pretreated their animals with indomethacin, an inhibitor of prostaglandin synthesis.<sup>(81)</sup> They found that the ability of ethanol to depress acid secretion was antagonized, suggesting that an increase in prostaglandin synthesis may mediate, in part, this effect of ethanol. However, they did not measure cyclic AMP levels, so conclusions on an interaction of prostaglandins and cyclic AMP in this system cannot be made, although cyclic AMP levels have been reported to be elevated in rat fundic muscle after intraperitoneal injection of prostaglandin E<sub>1</sub>.<sup>(82)</sup> Also, it is unfortunate that these experiments were not done in dogs, where the mechanism of the inhibition of acid secretion at high ethanol concentrations is less clear.

In summary, the effects of ethanol on gastric acid secretion are accompanied by changes in superficial mucosal cyclic AMP levels. These changes reflect, in part, alterations in the activities of adenylate cyclase and phosphodiesterase. It is not clear how ethanol exerts its effect, but considering the high ethanol concentrations used, partial denaturation or structural alterations of the enzymes are possible. Also, part of the reduction in acid secretion that can be induced by ethanol in some species might be mediated through a reduction in ATP levels or an increased synthesis and release of prostaglandins. It would be interesting to determine whether ethanol actually stimulates prostaglandin release and whether it is more effective in species where ethanol has a pronounced inhibitory effect—for example, in the rat. At this stage, no cause-and-effect relationship has been established between the ethanol-induced reduction of acid secretion and prostaglandins. Further research will be required to clarify this point.

Under normal circumstances where systemic effects of ethanol are considered, the high concentrations of 1–50% used in the experiments with the stomach are far in excess of what would be compatible with life. However, we are dealing with localized actions of ethanol under conditions similar to normal drinking of alcoholic beverages. Taking into account the damage to the stomach known to result from high concentrations of ethanol,<sup>(62)</sup> the preceding discussion further emphasizes the need for caution in drinking beverages with a high alcoholic content.

#### D. ETHANOL AND CYCLIC NUCLEOTIDES IN OTHER TISSUES

##### 1. Liver

Compared to the central nervous system and the stomach, very little research has been done on the possible involvement of cyclic nucleotides in the actions of ethanol on other organs. A few reports exist but generally are not enlightening as to their possible significance.

Ethanol has been reported to induce alterations in cyclic AMP metabolism in the liver. Short-chain aliphatic alcohols stimulate glucagon-responsive adeny-

ate cyclase.<sup>(83)</sup> The potency increases with each additional carbon in the chain. However, no differences in potency are observed among primary, secondary, and tertiary butanols. The enhanced activity of adenylate cyclase by ethanol is not apparent until the concentrations are at least 2%, with the maximum effect reached at 5% ethanol. These alcohols, also, stimulate adenylate cyclase *in vitro* in other tissues, including kidney, intestine, fat, and brain.<sup>(84,85)</sup> In these studies only unphysiologically high concentrations could elicit the response.

Because of the peripheral adrenergic hyperactivity observed after chronic ethanol administration,<sup>(37,86)</sup> French *et al.*<sup>(87)</sup> explored the possibility that similar treatment might alter the sensitivity of the cyclic AMP system to norepinephrine in the liver. They found that after 18 weeks of ethanol ingestion, the ED<sub>50</sub> for norepinephrine stimulation had increased sixfold in liver homogenate particulate fractions, indicating the development of adrenergic subsensitivity. This altered sensitivity returned to control values 3 days after withdrawal, unlike that observed in brain.<sup>(40)</sup> However, if the measurements were made in liver mitochondria, an increased sensitivity was seen.<sup>(88)</sup> Finally, the effects of chronic ethanol treatment appear to be mediated through  $\beta$ -receptors, since  $\beta$ -adrenergic antagonists block the ability of norepinephrine to stimulate cyclic AMP accumulation, with no effect by  $\alpha$ -adrenergic antagonists.<sup>(87)</sup>

## 2. Skin

One study has appeared studying the effect of short-chain alcohols on cyclic AMP in the skin. Yoshikawa *et al.*<sup>(89)</sup> reported that cyclic AMP levels are increased up to 2.5-fold in a concentration-dependent manner in epidermal slices incubated in 1–5% ethanol. Similar results were found with 1-propanol and acetone, but not with methanol and 1-butanol. This effect appears to be related to an activation of epidermal adenylate cyclase. As yet, no physiological significance can be attributed to these findings.

## E. SUMMARY AND CONCLUSIONS

It is now clear that acute and chronic treatment with ethanol exerts a number of effects on the cyclic nucleotides in several organ systems. In the brain, for instance, ethanol intoxication results in lower levels of cyclic nucleotides, especially cyclic GMP. However, after chronic administration of ethanol, indicators of cyclic nucleotide function are elevated. These changes appear to reflect changes in the activity of the nervous system. During intoxication, the brain is depressed, whereas during ethanol withdrawal syndrome, hyperactivity can be observed. It is yet to be determined whether changes in cyclic-nucleotide-mediated systems are responsible for intoxication, dependence, or other biological alterations for which they have been implicated after ethanol consumption. At the present state of research, changes in cyclic nucleotides can be shown to



be concomitants of certain effects of ethanol. However, no one has been able to demonstrate that these changes in themselves can explain ethanol-induced behavioral abnormalities. In fact, because of the effect ethanol has on neurotransmitters and their relationship to cyclic nucleotides, it is possible that alterations in cyclic nucleotides are secondary to some other response of ethanol. The exact role of cyclic nucleotides in the actions of ethanol and its potential significance will require further research.

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