INTRODUCTION

The purpose of this manuscript is to describe a special interaction of the ethanol metabolite, acetaldehyde, with adrenergic systems. The term "adrenergic systems" is taken to comprise all catecholamine-containing nerve tracts, both in the central nervous system and in the peripheral sympathetic system; additionally, the adrenal medulla is included, as are certain of the clusters of interneurons in sympathetic ganglia (e.g. the dopamine-containing interneurons).

It has been established (1) that catecholamines within intact cells can condense spontaneously with acetaldehyde or with formaldehyde (a metabolite of methanol) to yield a group of ring-closed products which contain the 1,2,3,4-tetrahydroisoquinoline (TIQ) nucleus (Figure 1).

Fig. 1. Route of synthesis of 6,7-dihydroxy-TIQs from catecholamines in adrenergic systems. Acetaldehyde, R3=CH3; formaldehyde, R3=H. Dopamine, R1=R2=H; norepinephrine, R1=OH, R2=H; epinephrine, R1=OH, R2=CH3.

These alkaloid products contain hydroxyl groups in the 6- and 7-positions, arising from the catechol portions of the corresponding catecholamines. A requirement for the ring-closure reaction to proceed at neutral pH is the presence of an activating hydroxyl group in the 3-position (the meta position) of the catecholamine (2). The hydroxyl group in the corresponding 4-position (the para position) does not strongly influence the ring-closure reaction. Thus, m-tyrosine and m-tyramine will condense spontaneously, but the naturally-occurring p-tyrosine and p-tyramine will not. For purposes of organic synthesis under laboratory conditions, the reactions of phenylethylamines without activating meta hydroxyl groups can be catalyzed by heat and strong acid (3).

The formation of TIQs in the adrenal medulla during perfusion of isolated cow adrenal glands with acetaldehyde or formaldehyde has been studied in detail (1,4-7). There is evidence for
biosynthesis of TIQs in the adrenals of methanol-intoxicated rats (8). Recently, Collins and Bigdeli (9) demonstrated the presence of one TIQ (salsolinol, formed by the condensation of dopamine with acetaldehyde) in the brains of rats treated with ethanol in combination with other drugs. Some studies have also been conducted with a purely in vitro system consisting of broken cell preparations (homogenates) of brain (10).

Because TIQs possess catecholamine-like structures, their interaction with adrenergic mechanisms is not unexpected (1). The numerous interactions of TIQs with adrenergic neurons were the subject of a recent review (11) (see also other articles in this volume). TIQs block uptake of catecholamines in vitro and in vivo. TIQs are themselves taken up and accumulated within the catecholamine-storage vesicles. They can be secreted from cells by calcium-dependent mechanisms. TIQs also appear to interact with catecholamine receptors: both agonist and antagonist actions have been noted. Some of these properties of TIQs are described in greater detail in this chapter.

SYNTHESIS OF TIQ ALKALOIDS AND RELEASE FROM THE ADRENAL MEDULLA BY ACETYLCHOLINE

Retrograde perfusion of the catecholamine-rich, cow adrenal gland with solutions of acetaldehyde or formaldehyde results in the synthesis of TIQ derivatives of epinephrine and norepinephrine. TIQ products within the adrenal medulla were observed routinely after perfusion with solutions containing 2.3 mM acetaldehyde (100 pg/ml) (1). Radiotracer studies with 14C-acetaldehyde showed that small amounts of TIQ products were formed when 23 pM acetaldehyde (1 pg/ml) was used (12); this latter concentration is in the range seen in human subjects during ethanol intoxication (13). When a relatively high concentration of formaldehyde was used (33 mM; 1 mg/ml), total conversion of adrenal catecholamines to TIQs was observed (4); such large conversions were not achieved at correspondingly high concentrations of acetaldehyde. The relatively greater extent of reaction of catecholamines with formaldehyde most probably results from a very much greater rate of reaction of formaldehyde compared to acetaldehyde (1).

The TIQs formed in the adrenal gland are bound in the catecholamine-storage vesicles (chromaffin granules) (14). Recent studies by Schneider (7) with 14C-acetaldehyde have shown that 14C-TIQs account for over 95% of the soluble 14C found in adrenal chromaffin granules. Other investigators (15) have established that release from the adrenal medulla takes place by exocytosis. In this process, which requires calcium ions, the chromaffin granules appear to fuse with the outer cell membrane and then they release their entire soluble contents, which include the catecholamines, ATP, dopamine beta-hydroxylase, and chromagranin (a binding protein).
Because TIQs are constituents of the chromaffin granule in acetaldehyde-perfused glands, it appeared likely that stimulation of such glands would lead to the release of the TIQs along with the catecholamines. Studies were undertaken to test this possibility (5):

Paired glands were perfused for one hour with either Tyrode’s solution (controls) or 23 mM acetaldehyde in Tyrode’s solution (experimental glands). The relatively high concentration of acetaldehyde was used to ensure a reasonable yield of TIQs in order to facilitate analyses by thin-layer chromatography. The glands were rinsed by perfusion with fresh solution without acetaldehyde for an additional hour. Subsequently, stimulation by perfusion with Tyrode’s solution containing 0.16 mM acetylcholine for two minutes, resulted in release of catecholamines from the control gland, and catecholamines plus TIQs from the experimental gland (Figure 2).

**Fig. 2.** Thin-layer chromatographic assays of Al(OH)₃-purified extracts of the perfusates from intact cow adrenal glands. Thin-layer chromatography was run in an upward direction on plates composed of Silica Gel G; the solvent was sec-butanol: formic acid: water (15: 3: 2). Plates were sprayed with K₃Fe(CN)₆ followed by FeC₁₃, which produced the intensely-colored Prussian blue wherever reducing agents (catechols) were present on the plate. The method is sensitive to about 0.1 μg catecholamine or TIQ. For further details, see reference 5. The figure shows paired adrenal glands before and during stimulation with 0.16 mM acetylcholine. Control gland, perfused with saline alone: (1) prior to and (2) during stimulation with acetylcholine. Acetaldehyde-perfused gland: (3) prior to and (4) during stimulation with acetylcholine. For structures of TIQ₁ (norepinephrine condensed with acetaldehyde) and TIQ₂ (epinephrine condensed with acetaldehyde) refer to figure 1.

Similar observations were made with carbachol (carbamylcholine) as the secretagogue.

The secretion process for TIQs, like that for catecholamines, was dependent upon calcium ions. When glands were stimulated with carbachol in the absence of Ca²⁺, neither the catecholamines nor the TIQs were found in the effluents from the glands. Secretory responses
were restored by replenishing Ca\textsuperscript{2+} to the gland. No secretion of catecholamines or TIQs was evident in the presence of 0.1 mM tetracaine, an agent that prevents the inflow of Ca\textsuperscript{2+} into stimulated glands. Thus, it appeared that the secretion of TIQs and catecholamines took place by the same process. In more recent studies, Rahwan et al. (6) confirmed and extended these observations; these investigators also noted that in the absence of Ca\textsuperscript{2+}, acetaldehyde by itself evoked the release of catecholamines, while the TIQs were retained within the gland.

**UPTAKE, STORAGE AND RELEASE OF TIQs BY PERIPHERAL SYMPATHETIC NERVES**

The probability that TIQs would enter adrenergic nerve terminals of the brain was established by the observation that TIQs interfered with (blocked) the uptake of 3H-catecholamines (16). More recently, blockade of 3H-catecholamine uptake by TIQs and some other alkaloids was studied in greater detail (17,18). The results of these and other studies indicate that TIQ alkaloids and 3H-catecholamines compete for transport sites on the axonal membrane. Studies with H-labelled TIQs have shown that some TIQs are actively taken up and accumulated by adrenergic nerve terminals of the brain in vitro (16) and by sympathetic nerve terminals of the heart, iris and sub-maxillary gland of mice or rats in vivo (19).

In the studies described below, fluorescence microscopy was used to study the uptake, storage and release of TIQs by the peripheral sympathetic nerve plexus in the rat and mouse iris. Formaldehyde-derived TIQs are intermediates in the well-known method for the visualization of catecholamines in tissues by means of fluorescence microscopy (reviewed by Corrodi and Jonsson, [20]). In this method, tissues are heated with formaldehyde gas under carefully defined conditions of humidity and temperature. The catecholamines first condense with formaldehyde to form TIQs, which become further transformed to fluorescent 3,4-dihydroisoquinolines (Figure 3).

![Figure 3: Dopamine (I) condenses with formaldehyde to form 6,7-dihydroxy-TIQ (II). In the formaldehyde condensation method for the visualization of dopamine HO under the fluorescent microscope, II is further transformed to fluorescent tautomers of the corresponding dihydroisoquinoline (III, IV).](image)

Since TIQs are reaction intermediates, it follows that TIQs in tissues can be visualized by the same procedure. Fluorescence microscopy was used to study the uptake and release of these alkaloids by peripheral adrenergic nerves. However, in order to avoid interference from...
fluorescence of endogenous norepinephrine, the animals were first treated with reserpine or a catecholamine synthesis inhibitor in order to deplete the endogenous norepinephrine.

In studies in vitro (21), norepinephrine depleted irides were incubated for thirty minutes at 37 degrees C in isotonic buffer containing norepinephrine, dopamine or 6,7-dihydroxy-TIQ (1-10 pg/m1). Fluorescence microscopy revealed that 6,7-dihydroxy-TIQ was taken up into the adrenergic plexus of the iris and that it was particularly well accumulated in the varicosities (nerve terminals), even in reserpinized preparations. TIQ accumulation was better than that for dopamine, but about 1/10th that for norepinephrine, as judged by fluorescence microscopy. Uptake was completely blocked by 10-5M desmethylimipramine. Studies performed by electron microscopy (22) showed that 6,7-dihydroxy-TIQ was stored in the catecholamine-binding vesicles of adrenergic nerve terminals in the iris and pineal gland.

In studies in vivo (23), rats were pretreated with alpha-methyl-p-tyrosine methyl ester (500 mg/kg). These animals showed very little evidence of an adrenergic nerve plexus in the iris (Figure 4a) due to depletion of endogenous norepinephrine. Under urethane anesthesia (2 g/kg), 6,7- dihydroxy-TIQ (10 mg/kg) was injected into the femoral vein in order to load the peripheral sympathetic nerve terminals with TIQ. Both cervical sympathetic trunks were then cut, and one trunk was stimulated at parameters that were supra-maximal for a normal animal (6 V amplitude, 2 msec duration, 15 biphasic pulses per sec). After thirty minutes of stimulation, the irides were removed and examined by fluorescence microscopy. The control iris (unstimulated) exhibited a rich adrenergic nerve plexus due to the presence of 6,7-dihydroxy-TIQ (Figure 4b); there were prominent varicosities and the overall appearance was similar to a normal iris filled with norepinephrine. In contrast, the iris that had been subjected to preganglionic stimulation showed very much less fluorescence intensity, with smaller varicosities and a smoother overall appearance (Figure 4c). The depletion was even greater when desmethylimipramine was used to prevent reuptake of released TIQ.

Fig. 4. Fluorescence microscopy was used to study the adrenergic nerve plexus in stretch preparations of irides from norepinephrine-depleted rats (treated with alpha-methyl-p-tyrosine methyl ester, 500 mg/kg). (A) Control iris. (B) After injection of 6,7 -dihydroxy-TIQ (10 mg/kg); a rich adrenergic plexus is visible. The plexus is indistinguishable from a normal plexus containing norepinephrine. (C) The iris that had been subjected to preganglionic stimulation and high-frequency stimulation after prior injection of TIQ.
norepinephrine. (C) Depletion of 6,7-dihydroxy-TIQ evoked by stimulation of the sympathetic trunk (see text).

AGONIST AND ANTAGONIST ACTIONS OF TIQS

Various investigators have demonstrated the ability of some complex TIQs, such as tetrahydropapaveroline, to activate beta-adrenergic receptors in the bronchioles (24) and in the fat pad (25) or isolated fat cells (18); in these studies, dilation of the bronchioles (24) or release of free fatty acids or glycerol (18,25) was observed. In the studies in which the release of 6,7-dihydroxy-TIQ from nerve terminals was evoked by electrical stimulation of the sympathetic trunk (see above), direct agonist actions of this TIQ on alpha-adrenoreceptors were noted (23). The specific actions that were observed were retraction of the eyelid accompanied by a pronounced protrusion of the eyeball and pupillary dilation (constriction of the iris). Such effects are typical for release of the natural transmitter, norepinephrine. However, norepinephrine was absent due to prior treatment with a catecholamine-synthesis inhibitor, and it was established that 6,7-dihydroxy-TIQ had not been transformed to norepinephrine within the nerve plexus. Because dose-response relationships (viz, magnitude of electrical stimulation vs. end organ response) were not studied, it is difficult to fully evaluate the efficacy of 6,7-dihyroxy-TIQ as a surrogate neurotransmitter in this system. However, it is noteworthy that responses to electrical stimulation were absent just prior to intravenous injection of the TIQ and that the final response to stimulation was indistinguishable from that in a normal rat. It is not clear from these studies whether 6,7-dihydroxyTIQ is weaker, stronger or equal to norepinephrine as a transmitter agent for each of the observed parameters of action.

Recent studies have investigated the effects of TIQs on dopamine receptors of the brain. Both agonist actions (stimulation of adenyl cyclase) (26) and antagonist actions (27) were observed. As a dopamine agonist, 6,7-dihydroxy-TIQ was approximately 1/10th as effective as apomorphine, and 1/100th as effective as dopamine (26). Salsolinol, on the other hand, inhibited the rise in cyclic AMP (27).

In a recent study, the effects of two dopamine-derived TIQs (6,7-dihydroxy-TIQ and salsolinol) on the rat vas deferens were investigated (28). The vas deferens with attached hypo-gastric nerve was mounted in an organ bath and superfused with modified Krebs' solution at a flow rate of 5 ml/min. The hypogastric never was stimulated at a voltage just supra-maximal for each preparation. Stimulation for thirty seconds was repeated every three minutes (one msec duration, twenty pulses per sec). Preparations were first stimulated to record baseline responses. They were then incubated for thirty minutes with TIQ (6 x 10-5M) and subsequently washed with fresh Krebs' solution without TIQ for fifteen minutes. The vas deferens was then
retested. In control preparations, responses were unaltered. However, in TIQ-treated preparations, changes in mechanical responses were observed. 6,7-dihydroxy-TIQ suppressed the initial twitch. Additionally, there was a transient potentiation of the second-phase response. Potentiation of the second-phase response could be maintained, however, if 6,7-dihydroxy-TIQ was present in the bathing fluid throughout. S(-)-Salsolinol, on the other hand, markedly suppressed both the twitch and second-phase responses (Figure 5). These actions of 6,7-dihydroxy-TIQ or S(-)-salsolinol could not be produced by incubating the vas deferens with dopamine or with tyramine (a norepinephrine-releasing agent). Therefore, altered responses after exposure to TIQs were not mediated by catecholamines.

The mechanisms for the actions of the TIQs on the vas deferens are not clear. Swedin (29) has noted that the twitch response is inhibited by prostaglandins released during nerve stimulation; perhaps the TIQs increase the release of prostaglandins or augment their effectiveness. On the other hand, diminished responses may imply receptor blockade; therefore a blocking action on norepinephrine-receptors should be considered.

**INHIBITION OF MONOAMINE OXIDASE (MAO) BY TIQS**

Yamanaka (30) and Collins et al. (31) showed that two TIQs, salsolinol and tetrahydropapaveroline, were weak inhibitors of MAO in homogenates of rat brain or liver. When serotonin was used as the substrate for MAO, Ki values in the range 0.14-0.35 mM were reported. However, TIQs sequestered in nerve terminals can be present at a relatively high molar concentration. Therefore, a relatively strong inhibition of intraneuronal MAO by TIQs was a distinct possibility.

More recent studies have tested the actions of TIQs within an intact peripheral adrenergic nerve plexus in vitro (32) and in vivo (33). In these studies, a reserpinized model was used. It is known that reserpinized nerves lose their capacity to retain 3H-norepinephrine. Although transport into nerves is apparently normal, the vesicular storage mechanism is blocked and cytoplasmic 3H-norepinephrine is subjected to degradation by mitochondrial MAO. In normal animals, the binding of NE in vesicles prevents degradation by MAO. Inhibition of MAO can
8. A MECHANISM FOR ALTERED ADRENERGIC ACTIVITY IN ALCOHOLISM

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protect cytoplasmic norepinephrine and, thereby, increase the amount of norepinephrine retained within the neuron. However, MAO inhibitors cannot normalize the retention of norepinephrine because access to storage vesicles is blocked in reserpinized animals.

Results of in vivo studies (33) are shown in Table 1. In these experiments, mice received injections of reserpine (10 mg/kg). Eighteen hours later, 6,7-dihydroxy-TIQ (10 mg/kg) was injected intravenously. Injection of this dose results in the accumulation of 6,7-dihydroxy-TIQ within nerve terminals of sympathetic nerves (23). Twenty minutes later, 3H-norepinephrine (100 pCi/kg) was injected intravenously. Exactly five minutes later, the hearts were removed and analyzed for 3H-norepinephrine and 3H-deaminated catechols. Some mice were treated additionally with desmethylimipramine (DMI, 20 mg/kg) at 1.5 hours prior to i.v. 3H-norepinephrine; DMI blocks the axonal membrane pump for amines and denies entry of 3H-norepinephrine (as well as 6,7-dihydroxy-TIQ) into adrenergic nerve terminals. The levels of 3H present specifically in the Fig. 5. The normal response of a rat vas deferens (left) consists of a rapid twitch, followed by a adrenergic nerve plexus of the heart was given by the difference between groups of animals with and without pretreatment with DMI.

| TABLE 1 | Accumulation of 6,7-Dihydroxy-TIQ as an Inhibitor of Extraneuronal Monoamine Oxidase Within the Adrenergic Nerve Plexus of the Mouse Heart |
|---|---|---|---|---|---|---|---|---|
| | Contaminate | DPM/mg | Contaminate | DPM/mg | Contaminate | DPM/mg |
| Reserpinated | 50 | 55 ± 3 |
| Reserpinated + 6,7-Dihydroxy-TIQ | 50 | 97 ± 3 |

a. The radioactivity present in non-neuronal sites in reserpine-treated groups was as follows: 3H-norepinephrine = 47 + 4 DPM/mg with and without TIQ, and 3H-deaminated catechols = 7 + 3 DPM/mg with, and 6 + 1 DPM/mg without TIQ.

b. p < 0.01 compared to the reserpine group.

The results of Table 1 show that 6,7-dihydroxy-TIQ raised the accumulation of 3H-norepinephrine from 8.8% of control to 37.4% of control within the adrenergic nerve plexus. Simultaneously, there was some decrease in levels of 3H-deaminated catechols. The ratio 3H-norepinephrine/3H-deaminated catechols rose from 0.74 in reserpinized mice to 4.17 in corresponding mice that received i.v. 6,7-dihydroxy-TIQ. These results are comparable to those reported for other MAO inhibitors in similarly designed studies (34,35) and they illustrate the effectiveness of 6,7-dihydroxy-TIQ as an inhibitor of MAO within an adrenergic nerve plexus in vivo. Similar studies, conducted in vitro with isolated mouse atria (32) have shown that
salsolinol is also an effective MAO inhibitor.

CONCLUSIONS

The foregoing experiments illustrate the range of interactions with adrenergic systems that may be anticipated for TIQs formed endogenously in a spontaneous reaction between acetaldehyde and neuronal catecholamines during intake of alcoholic beverages. TIQs are capable of interfering with mechanisms that regulate the synaptic properties of catecholamines and they, themselves, can be stored and then released from nerves. Upon discharge from nerve terminals TIQs can function either as direct agonists (surrogate transmitters) of adrenergic receptors, or as antagonists to the catecholamines, depending upon the specific receptor area and specific TIQ in question. Because TIQs can be taken back up into nerve terminals and sequestered, and because they are not metabolized by MAO, it is easy to imagine how they might represent a chemical residuum of alcohol persisting into post-intoxication states. Further research is required to determine whether or not the behavioral responses seen during alcohol intoxication or withdrawal are based, in part, on the physiologic actions of small quantities of TIQs that alter the interactions between catecholamine-containing nerve terminals and their receptors.

REFERENCES

8. A MECHANISM FOR ALTERED ADRENERGIC ACTIVITY IN ALCOHOLISM

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