ALCOHOL AND CATECHOLAMINE DISPOSITION:

A ROLE FOR TETRAHYDROISOQUINOLINE ALKALOIDS

GERALD COHEN
College of Physicians & Surgeons of Columbia University, 640 West 168th Street, New York, New York 10032, U.S.A.

INTRODUCTION

THE PLANT alkaloids comprise a broad grouping of compounds (ROBINSON, 1968), many of which exhibit drug-like actions. A subclassification is the tetrahydroisoquinoline (TIQ) group (SHAMMA, 1972), which contains alkaloids that appear to be derived from dopamine (DA). Some of the more complex alkaloids such as morphine and codeine originate from DA and pass through a benzyl-TIQ as a biosynthetic intermediate (KIRBY, 1967). Plant alkaloids related to norepinephrine (NE) or epinephrine (E) are unknown, presumably because plants do not β-hydroxylate the DA side chain. Recently, laboratory synthesis of an NE-derived TIQ was achieved by COLLINS and KERNOZEK (1972).

The main thesis of this paper is that a group of TIQ alkaloids can be biosynthesised in people when they drink alcoholic beverages. These substances might interfere with normal adrenergic neurotransmission in the brain and in the periphery and, by so doing, they might be responsible for alterations in mood, mentation and behaviour.

PATHWAY OF BIOSYNTHESIS OF TIQ ALKALOIDS

The work of SCHÖPF and BAYERLE (1934) showed that a spontaneous condensation occurred with DA and acetaldehyde under conditions amenable to plant life (neutral pH, ambient temperature). The presence of an activating hydroxyl group opposite (para) to the point of ring closure facilitates the formation of the TIQ ring system at neutral pH (ZENKER, 1966). All catecholamines can undergo this reaction and a variety of aldehydes can be used. For example, the condensation of dopa with pyridoxal phosphate (vitamin B_6, an enzyme cofactor) causes substrate inhibition of dopa decarboxylase (3,4-dihydroxy-L-phenylalanine carboxy-lyase, E.C. 4.1.1.26) (SCHOTT and CLARK, 1952).

A pathway for the biosynthesis of TIQ alkaloids in people becomes evident. During ethanol intoxication, acetaldehyde circulates in the bloodstream (MAJCHROWICZ and MENDELSON, 1970). It seems likely that this acetaldehyde would condense with DA, NE and E in their storage sites in the brain and in the periphery to form TIQ derivatives. SANDLER and coworkers (1973) recently reported that administration of ethanol to Parkinson patients who were being treated with large doses of L-dopa, provoked urinary excretion of the TIQ condensation product of DA with acetaldehyde.

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

Retrograde perfusion of isolated, fresh cow adrenal glands with solutions of acetaldehyde or formaldehyde results in the synthesis of TIQ derivatives of E and NE

(COHEN and COLLINS, 1970). These TIQs are bound, in part, to the chromaffin granules (GREENBERG and COHEN, 1972). With 33 mM formaldehyde (1 mg/ml), a total conversion of catecholamines to TIQs is achieved within one hour (COHEN, 1971a); such large conversions have not been observed with acetaldehyde. With 23 μM acetaldehyde (1 μg/ml), which is in the concentration range reported in the blood of persons ingesting alcoholic beverages, some formation of TIQs has been detected (COHEN, 1971b). There is evidence for synthesis of formaldehyde-derived TIQs in the adrenals of rats receiving i.p. injections of methanol (COHEN and BARRETT, 1969).

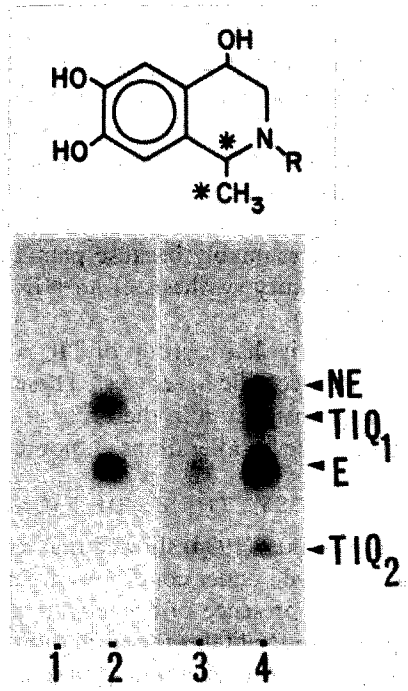

FIG. 1.—Thin-layer chromatographic assays of $Al(OH)_3$-extracts of perfusates of cow adrenal glands. Shown are paired glands prior to stimulation (1, control; 3, acetaldehyde-perfused) and during stimulation with acetylcholine (2, control; 4, acetaldehyde-perfused). TIQ_1 = NE derivative (R = H); TIQ_2 = E derivative ($R = CH_3$). The acetaldehyde portion of the TIQ structure is shown by asterisks.

Release of acetaldehyde-derived TIQs from cow adrenal glands was recently studied (GREENBERG and COHEN, 1973). Paired glands were perfused for 1 hr with either Tyrode's solution (controls) or with 23 mM acetaldehyde (1 mg/ml) in Tyrode's solution (experimental glands). The relatively high concentration of acetaldehyde was used to obtain 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 2 min, resulted in release of catecholamines from the control gland, and catecholamines plus TIQs from the experimental gland (Fig.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 Ca^{2+}, neither the catecholamines nor the TIQs were found in the perfusates. Secretory responses were restored by replenishing Ca^{2+} to the gland. No secretion of catecholamines or TIQs was evident in the presence of 0·1 mM tetracaine, an agent which prevents the inflow of Ca^{2+} into stimulated glands. Since TIQs are bound to the chromaffin granules and since they are released along with catecholamines in a Ca^{2+}-dependent process, it appears that the secretion of TIQs and catecholamines takes place by the same process.

PTAKE AND RELEASE OF TIQS BY PERIPHERAL SYMPATHETIC NERVES

The formaldehyde-derived TIQs are intermediates in the well-known method (reviewed by CORRODI and JONSSON, 1967) for the visualisation of catecholamines in tissues by means of fluorescence microscopy. 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. Since TIQs are reaction intermediates, it follows that TIQs in tissues can be visualised 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 NE, the animals were first treated with reserpine or α-methyl-*p*-tyrosine methyl ester to deplete the catecholamines.

In *in vitro* studies (COHEN, MYTILINEOU and BARRETT, 1972), irides from reserpinised or α-methyl-*p*-tyrosinised rats were incubated for 30 min at 37°C in isotonic buffer containing NE, DA or 6,7-dihydroxy-TIQ (1–10 μg/ml). Fluorescence microscopy revealed that the 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 reserpinised preparations. TIQ accumulation was better than that for DA, but about 1/10th that for NE, as judged by fluorescence microscopy. Uptake was completely blocked by 10^{-5} M desmethylimipramine.

In recent studies (MYTILINEOU, COHEN and BARRETT, 1973), rats were treated with α-methyl-*p*-tyrosine methyl ester (500 mg/kg). These animals showed very little evidence of an adrenergic nerve plexus in the iris (Fig. 2a) due to depletion of endogenous NE. Under urethane anaesthesia (2 g/kg), 6,7 dihydroxy-TIQ (10 mg/kg) was injected into the femoral vein. Both cervical sympathetic trunks were cut and then one trunk was stimulated at parameters which were supramaximal for a normal animal (6 V amplitude, 2 msec duration, 15 biphasic pulses per sec). After 30 min 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 (Fig. 2b). There were prominent varicosities and the overall appearance was similar to a normal iris filled with NE. In contrast, the iris that had been subjected to preganglionic stimulation showed diminution in fluorescence intensity, with smaller varicosities and a smoother overall appearance (Fig. 2c). The depletion was even greater when desmethylimipramine was used to prevent reuptake of released TIQ.

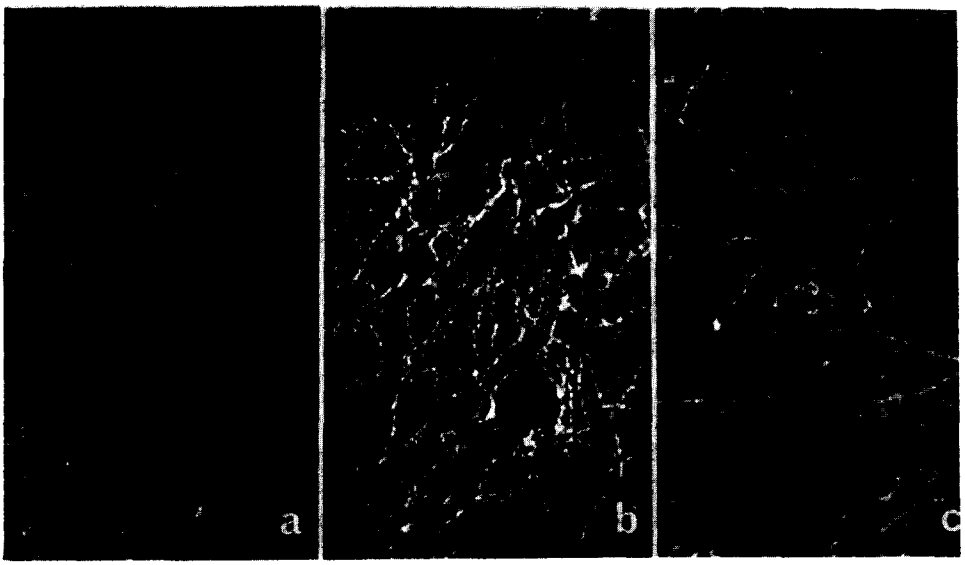

FIG. 2.—Formaldehyde vapour-treated stretch preparations of irides from NE-depleted rats. Control (a) and injected with 6,7-dihydroxy-TIQ (b, c). The unstimulated (b) and stimulated (c) irides are from the same animal.

During stimulated release of 6,7-dihydroxy-TIQ, there was marked retraction of the eyelid and protrusion of the eyeball. Dilatation of the pupil was also evident. These responses were similar to those seen in normal (NE-containing) animals. These responses were absent just prior to the injection of the TIQ.

CONCLUSIONS

Catecholamines in tissues can condense with acetaldehyde (ethanol metabolite) or formaldehyde (methanol metabolite) to form a series of hydroxylated TIQ alkaloids. The TIQs possess the following properties:

(1) They are bound to vesicles in the adrenal medulla (GREENBERG and COHEN, 1972) and in peripheral adrenergic nerves (TENNYSON *et al.*, 1973).

(2) They can be taken up into peripheral adrenergic nerve terminals both *in vitro* (COHEN, MYTILINEOU and BARRETT, 1972) and *in vivo* (LOCKE, DEMBIEC and COHEN, 1973) by means of a cocaine-sensitive and desmethylimipramine-sensitive process.

(3) They can be taken up *in vitro* into nerve endings in the brain and they block the uptake of catecholamines (HEIKKILA, COHEN and DEMBIEC, 1971). When injected into the brain, they release catecholamines and cause hypothermia (BREZENOFF and COHEN, 1973).

(4) They can be released from tissues by exposure directly to acetylcholine GREENBERG and COHEN, 1973) or by preganglionic stimulation.

(5) When released by preganglionic stimulation, 6,7-dihydroxy-TIQ can interact with adrenergic receptors in the eye. It was recently reported (LEE *et al.*, 1973) that 1-methyl-6,7-dihydroxy-TIQ (salsolinol) activates adrenergic receptors in rat adipose tissue (cf. also HJORT *et al.*, 1942).

These properties indicate that catecholamine-derived TIQ alkaloids can be classified as potential neurotransmitter agents in adrenergic systems. The criteria for classifying a compound as a false transmitter include uptake, storage and release of the substance (KOPIN, 1968). The TIQs possess these properties and, additionally, they can react with certain adrenergic receptors. The properties of the TIQs raise some interesting speculations:

Speculation No. 1.

Is it possible that some of the well-known actions of alcoholic beverages are dependent, in part, upon alterations in adrenergic neurotransmission produced by the presence of TIQ alkaloids in nerve terminals? Although present in small amount, these alkaloids can achieve a precision of action by their discharge from nerve terminals directly onto adrenergic receptor areas. Released TIQs might activate some receptors, block others, or interfere with a variety of enzymes that act on or interact with the catecholamines.

Speculation No. 2.

The TIQs possess a property that is attractive for a consideration of post-intoxication states. The TIQs are bound in vesicles and, therefore, they can be retained, perhaps for prolonged periods of time. Furthermore, they are not attacked by monoamine oxidase (monoamine:oxygen oxidoreductase, E.C. 1.4.3.4.) (unpublished observation) and they can be taken back up after discharge and "used" again. Many bodily changes (JAFFE, 1970) such as psychomotor agitation or "hangover" occur as the general sedative or depressant actions of alcohol are wearing off. Still others, such as tremulousness, hyperexcitability, hallucinosis and seizure, are seen after particularly heavy or long-term drinking, and they occur when blood alcohol levels have either declined considerably or are absent. Are some of these changes due, in part, to the persistent actions of TIQs remaining in nerve terminals and at receptor areas? Are TIQ actions masked during alcohol intoxication, but made more evident in the absence of sedative?

SUMMARY

The current work was undertaken to explore the possibility that TIQs may play a role during alcohol intoxication and/or in post-intoxication states. At the present time, the available data show only that the catecholamine-derived TIQs are interesting pharmacologic agents for manipulating aspects of catecholaminergic function. Much remains to be done to explore the potential actions of the TIQs and to delineate their role(s) in the bodily responses to alcohol.

Acknowledgement—Portions of this work were supported by Grants AA-00243 and NS-05184 from the U.S. Public Health Service. I am indebted to the following friends and associates for their contributions to this work: Dr. Robert Barrett, Dr. Henry Brezenoff, Dr. Michael Collins, Ms. Dorothy Dembiec, Dr. Robert Greenberg, Dr. Richard Heikkila, Dr. Steven Locke, Dr. Catherine Mytilineou, Dr. Virginia Tennyson.

REFERENCES

BREZENOFF H. E. and COHEN G. (1973) *Neuropharm.* In press.

COHEN G. (1971a) In: *Advances in Mental Science III. Biologic Aspects of Alcohol* (ROACH M. K., MCISAAC W. M. and CREAVEN P. J., Eds.) pp. 267–284, University of Texas Press, Austin.

COHEN G. (1971b) *Biochem. Pharmacol.* **20,** 1757–1761.

COHEN G. and BARRETT R. (1969) *Fed. Proc.* **28,** 288 Abs.
COHEN G. and COLLINS M. A. (1970) *Science (Wash.)* **167,** 1749–1751.
COHEN G., MYTILINEOU C. and BARRETT R. E. (1972) *Science (Wash.)* **175,** 1269–1272.
COLLINS M. A. and KERNOZEK F. J. (1972) *J. Heterocyclic Chem.* **9,** 1437–1440.
CORRODI H. and JONSSON G. (1967) *J. Histochem. Cytochem.* **15,** 65–78.
GREENBERG R. S. and COHEN G. (1972) *Eur. J. Pharmacol.* **18,** 291–294.
GREENBERG R. S. and COHEN G. (1973) *J. Pharmacol. Exp. Ther.* **184,** 119–128.
HEIKKILA R., COHEN G. and DEMBIEC D. (1971) *J. Pharmacol. Exp. Ther.* **179,** 250–258.
HJORT A. M., DE BEER E. J., BUCK J. S. and RANDALL L. O. (1942) *J. Pharmacol. Exp. Ther.* **76,** 263–269.
JAFFE J. H. (1970) In: *The Pharmacological Basis of Therapeutics* (GOODMAN, L. S. and GILMAN A., Eds.) 4th ed., pp. 291–293, Macmillan, New York.
KIRBY G. W. (1967) *Science* **155,** 170–173
KOPIN I. J. (1968) *Ann. Rev. Pharmacol.* **8,** 377–394.
LEE O. S., MEARS J. E., BARDIN J. J., MILLER D. D. and FELLER D. R. (1973) *Fedn. Proc.* **32,** 723 Abs.
LOCKE S., DEMBIEC D. and COHEN G. (1973) *Fedn. Proc.* **32,** 526 Abs.
MYTILINEOU C., COHEN G. and BARRETT R. E. (1973) *Fedn. Proc.* **32,** 739 Abs.
ROBINSON T. (1968) *The Biochemistry of Alkaloids.* Springer-Verlag, New York.
SANDLER M., CARTER S. B., HUNTER K. R. and STERN G. M. (1973) *Nature, Lond.* **241,** 439–443.
SCHÖPF C. and BAYERLE H. (1934) *Ann. Chem.* **513,** 190–202.
SCHOTT H. F. and CLARK W. G. (1952) *J. Biol. Chem.* **196,** 449–462.
SHAMMA M. (1972) *Organic Chemistry* 25, *The Isoquinoline Alkaloids: Chemistry and Pharmacology.* Academic Press, New York.
TENNYSON V. M., COHEN G., MYTILINEOU C. and HEIKKILA R. (1973) *Brain Res.* **51,** 161–169.
ZENKER N. (1966) *J. Med. Chem.* **9,** 826–828.