Effects of several catecholamine-derived tetrahydroisoquinolines on the hypogastric nerve-vas deferens preparation of the rat

A class of 1,2,3,4-tetrahydroisoquinolines (TIQs) can be formed by the spontaneous condensation of catecholamines with aldehydes, such as formaldehyde or acetaldehyde (Cohen & Collins, 1970; Yamanaka, Walsh & Davis, 1970; Cohen, 1971). Recently, Collins & Bigdeli (1975) reported the presence of small amounts of the dopamine-acetaldehyde condensation product, salsolinol, in rat brain after administration of ethanol, and Sandler, Carter & others (1973) reported that ethanol ingestion provoked urinary excretion of salsolinol in patients receiving L-dopa.

TIQs possess some of the properties of false or surrogate transmitters in adrenergic systems (see review by Cohen, 1973). Of particular relevance to the present study, it has been shown that TIQs can be taken up and accumulated by peripheral sympathetic nerve terminals, and released by subsequent preganglionic stimulation (Mytilineou Cohen & Barrett, 1974). The TIQs exhibit both agonist (Miller, Horn & Iversen, 1974; Mytilineou & others, 1974) and antagonist actions (Sheppard & Burghardt, 1974) on catecholamine receptors, depending upon the tissue preparation and TIQ derivative used. The ability of TIQs to release catecholamines from tissues has also been noted (Heikkila, Cohen & Dembiec, 1971; Brezenoff & Cohen, 1973).

The innervated vas deferens provides a convenient isolated tissue with a rich adrenergic nerve supply, and no spontaneous activity (Huković, 1961). Stimulation of the hypogastric nerve results in a stimulus-dependent release of noradrenaline from the tissue (Farnebo & Malmfors, 1971). Extensive studies of this preparation have shown that it is not, however, a "classical" adrenergically innervated preparation. Swedin (1971c) has demonstrated that stimulation of the hypogastric nerve for 30 s periods results in a biphasic contraction of the vas deferens, the second phase of which shows characteristics of a typical adrenergic response.

In the experiments described here, we used the isolated, innervated vas deferens of the rat as a test organ to further elucidate the pharmacologic properties of one noradrenaline-derived and two dopamine-derived TIQs. Drugs used were 6,7-dihydroxy-TIQ hydrobromide (prepared by Dr. S. Ginsburg, Columbia University; cf., Cohen & Collins, 1970), *S*(−)-salsolinol hydrochloride (gift from Dr. S. Teitel; cf. Teitel, O'Brien & others, 1972), 4,6,7-trihydroxy-TIQ (gift from Dr. M. Collins; cf. Collins & Kernozek, 1972), (−)-noradrenaline bitartrate (Winthrop), tyramine hydrochloride (Sigma), acetylcholine bromide (Eastman) and dopamine hydrochloride (Calbiochem).

Male Sprague-Dawley rats (250–350 g) were decapitated and the vasa deferentia dissected with a modification of the method of Huković (1961). Since the hypogastric nerve in the rat is fragile, it was not cleared of surrounding mesentery, nor separated from the accompanying blood vessel. Once isolated, the preparation was mounted in a 50 ml organ bath and superfused continuously with modified Krebs solution (Huković, 1961) at a flow rate of approximately 5 ml min^{-1}. The superfusion was stopped during incubation with drugs. The organ bath was maintained at 32° and was bubbled with 5% CO_2 in oxygen.

Mechanical activity was recorded according to the method of Farnebo & Malmfors (1971). The tissue was initially adjusted to a tension of 0·25 g and allowed to equilibrate for 15 min. The nerve was stimulated via platinum electrodes coupled via a Grass SIU-5 stimulus isolation unit to a Grass S-48 stimulator. Rectangular pulses of 1 ms duration were delivered at 20 s^{-1} (submaximal frequency) and at a voltage which was just supramaximal for each tissue preparation. The preparations were stimulated for 30 s every 3 min.

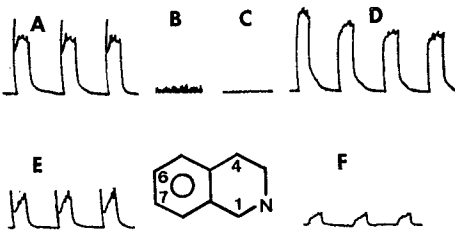

FIG. 1. Results of two experiments. The structure of 1,2,3,4-tetrahydroisoquinoline is shown and the 1,4,6 and 7 positions are marked. The chart speed was increased 4-fold during nerve stimulation. Recorded responses began promptly and ceased promptly upon application and termination of nerve stimulation (30 s). Experiment 1: A, control responses; B, during incubation with 6×10^{-5}M 6,7-dihydroxy-TIQ (no electrical stimulation); C, following washout of drug (no electrical stimulation); D, responses to electrical stimulation after drug treatment. Experiment 2: E, control responses; F, responses to electrical stimulation after incubation with and washout of *S*-salsolinol (1-methyl-6,7-dihydroxy-TIQ, 6×10^{-5}M). Note suppression of twitch and transient potentiation of second phase in experiment 1, and suppression of both twitch and second phase responses in experiment 2.

As previously reported by Swedin (1971a), stimulation of the hypogastric nerve-vas deferens preparation for 30 s produced a biphasic contraction (Fig. 1A). This consisted of an initial transient contraction ("twitch"), which was followed after about 5 s by a slower, more sustained contraction ("second phase"). In the present experiments, both phases of the response decreased progressively during the first several stimulations, but then reached a constant level. The steady response was taken as the control response. Each drug was tested on three tissue preparations.

6,7-Dihydroxy-TIQ (6×10^{-5}M, dopamine-formaldehyde condensation product) left in contact with the tissue for 30 min elicited small, random twitches, but the baseline tone of the preparation was not altered (Fig. 1B). On washing with fresh Krebs solution for 10 min followed by superfusion at the normal rate for a further 5 min, the original resting state was restored (Fig. 1C).

In contrast, the dopamine-acetaldehyde condensation product (*S*-salsolinol, 6×10^{-5}M) elicited twitches in only one of 3 preparations tested, while the noradrenaline-formaldehyde condensation product (4,6,7-trihydroxy-TIQ, 6×10^{-5}M) elicited no response during a 30 min incubation period. Incubation with tyramine (10^{-5}M) elicited small random twitches similar to those caused by 6,7-dihydroxy-TIQ and incubation with dopamine (6×10^{-5}M) markedly increased the baseline tone of the vas deferens and also elicited small random twitches.

Following incubation with drugs and subsequent washing, the responses to nerve stimulation were retested and compared to control. After such exposure to 6,7-dihydroxy-TIQ (6×10^{-5}M), the initial twitch was reduced or abolished, while the second phase contraction was transiently potentiated (Fig. 1D, cf. A). Potentiation of the second phase persisted for only 2–3 stimulations when the tissue was superfused with fresh Krebs solution. However, if 6×10^{-5}M TIQ was added to the superfusing solution, the potentiation was maintained. When the tissue was treated with *S*-salsolinol (6×10^{-5}M), subsequent stimulation of the hypogastric nerve elicited a response in which the twitch was markedly reduced or abolished and the second phase contraction was also considerably reduced (Fig. 1F, cf. E). The response elicited by nerve stimulation after treatment with *S*-salsolinol developed more slowly than did that observed after treatment with 6,7-dihydroxy-TIQ. In contrast to the effects observed with the two dopamine-derived TIQs, incubation with the noradrenaline derived compound 4,6,7-trihydroxy-TIQ (6×10^{-5}M) did not alter either phase of the contractile response.

In separate experiments, the vasa deferentia were cleared of surrounding mesentery,

nerves and blood vessels. They were then mounted in the organ bath and used to test the contractile effects of exogenously applied drugs. It was found that 6,7-dihydroxy-TIQ and 4,6,7-trihydroxy-TIQ had potencies of approximately 0·002 relative to that of noradrenaline in eliciting contractions.

The biphasic response of the vas deferens to stimulation of the hypogastric nerve is complex, but both phases appear to be adrenergic in character (see Swedin 1971a, c). The vas deferens is innervated by "short" adrenergic neurons with a peripheral ganglionic (cholinergic) relay located close to the target organ. The second stage of contraction shows characteristics of a classical adrenergic-effector junction: It is sensitive to catecholamine depletors (e.g., reserpine or α-methyl-*p*-tyrosine), to neuron blockers (e.g., guanethidine) and to α-adrenoceptor blocking agents (e.g., phenoxybenzamine). The "twitch" response is unusual in that it is potentiated by α-blockers, insensitive to β-blockers and resistant to change by pretreatment with reserpine or α-methyl-*p*-tyrosine.

Swedin (1971b) has demonstrated that the initial twitch is inhibited by release of a prostaglandin-like substance during nerve stimulation. Reduced or abolished twitch responses to nerve stimulation observed after incubation of the vas deferens with either 6,7-dihydroxy-TIQ or *S*-salsolinol may have thus resulted from increased release or effectiveness of prostaglandins.

Suppression of the second stage contraction in preparations treated with *S*-salsolinol is suggestive of receptor blockade. Blocking actions by both *S*- and *R*-salsolinol on central dopamine receptors have been reported (Sheppard & Burghardt, 1974), whereas 6,7-dihydroxy–TIQ exerts a direct agonist action in the same system (Miller & others, 1974).

Compounds having anticholinesterase activity are capable of potentiating stimulation-induced contractions in the vas deferens (Birmingham, 1966). However, such an explanation does not appear relevant to the potentiation of second phase contraction observed in the vas deferens treated with 6,7-dihydroxy-TIQ. We tested for anticholinesterase activity using the rat ileum: cumulative dose-response curves to acetylcholine were not altered by the presence of 6,7-dihydroxy-TIQ (6×10^{-5}M), suggesting that anticholinesterase activity probably did not play a role. The potentiation by 6,7-dihydroxy-TIQ is more likely related to the effects of this compound in blocking uptake of noradrenaline (Cohen, Heikkila & others, 1974), or inhibiting monoamine oxidase (Cohen & Katz, 1975; Collins, Cashaw & Davis, 1973), or perhaps to a direct agonist action.

Experiments in which the vas deferens was stimulated by indirect chemical means have led us to believe that the effects observed with 6,7-dihydroxy-TIQ and *S*-salsolinol cannot be mediated through release of noradrenaline. Prior exposure to tyramine (10^{-5}M) or to dopamine (6×10^{-5}M) did not alter the subsequent biphasic response to stimulation of the hypogastric nerve.

In view of the complication imposed by the presence of a ganglionic relay during stimulation, our results should be considered preliminary. Further studies with post-ganglionic stimulation may help to clarify the tentative interpretations of the data. Although the modes of action of the two dopamine-derived TIQs in the vas deferens are incompletely defined, it is evident that the tissue represents another system in which these compounds are able to modify nerve–effector cell function. Such a finding further supports the concept that if these compounds are formed in sufficient amounts from metabolic aldehydes (e.g., acetaldehyde, formaldehyde) during chronic ethanol intake, the resulting effects upon adrenergic function may be of pharmacological or neurological importance.

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