Turnover of noradrenaline in rat heart during the period of light at a room temperature of 23 ± 1 °C and 32 ± 1 °C resp.

Room temperature: 23 ± 1 °C			Room temperature: 32 ± 1 °C		
Half-life $t_{1/2}$ (h)	Rate constant k (h^{-1})	Turnover rate ($\mu g \times g^{-1} \times h^{-1}$)	Half-life $t_{1/2}$ (h)	Rate constant k (h^{-1})	Turnover rate ($\mu g \times g^{-1} \times h^{-1}$)
17.7	0.0392	0.035	11.0	0.0629	0.057
14.6	0.0475	0.045	11.7	0.0594	0.063
17.8	0.0389	0.038	8.5	0.0818	0.074
15.7	0.0442	0.040	10.3	0.0675	0.081
$\bar{X}$ 16.3(14.1–19.4)*	0.0425	0.039	10.2(8.3–13.3)*	0.0679**	0.069**
SEM	± 0.0020	± 0.002		± 0.0049	± 0.006

The parameters of the turnover were calculated from decay of the specific activity after i.v. injection of ^{3}H-(–)-noradrenaline. Mean values ± SEM of 4 separate experiments at either room temperature. *Geometric mean with 95% confidence limits. **p (control to heat) < 0.005.

Discussion. Until now no data are available demonstrating the effect of an elevated environmental temperature on the cardiac turnover of noradrenaline in the rat. The experimental results presented here clearly show that an acute increase in environmental temperature from 23°C to 32°C results in a significantly increased cardiac turnover of noradrenaline in the rat. Such an elevation in the room temperature leads to an increase in the rectal temperature[9], to an increased sympathetic nervous activity in some peripheral organs[12] and to an increase in the cardiac output mainly due to an elevated heart rate[10]. Thus, the increased cardiac turnover of noradrenaline on acute exposure to heat can be explained as a consequence of an accelerated sympathetic nervous activity. However, it remains unsolved whether the increased turnover is due to a specific effect of heat on the thermoregulatory centers[8], thereby increasing the peripheral sympathetic nervous system, or whether a non-specific stress reaction takes place[8].

The results presented here together with earlier findings demonstrating circadian variations in the cardiac turnover of noradrenaline[4] and the dopamine turnover in rat brain[13], further indicate the importance of exact standardization of experimental conditions in animal studies. The importance of a controlled thermal environment was already shown by Fuhrman and Fuhrman[14] and Weihe[15] who could demonstrate that variations in environmental temperature greatly influence the sensitivity of experimental animals to drugs, leading to variations of results in drug testing.

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Fluorescence histochemical demonstration of the uptake of dopamine-derived dihydroisoquinoline in the hypothalamic neurons

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Summary. The uptake and the accumulation of dopamine-derived fluorescent dihydroisoquinoline were demonstrated with direct fluorescence histochemistry in the hypothalamic dopaminergic neurons, in the nerves of the neurointermediate lobe, and in some endocrine cells of the hypophysis of the rat.

It has been suggested that the in vivo formation of tetrahydroisoquinolines (TIQs) derived from the condensation of catecholamines (dopamine, noradrenaline and adrenaline) with aldehydes produced in the metabolism of alcohols plays a part in the pathological manifestation of alcohol intoxication and dependence[1–3]. Furthermore, an increase in dopamine concentration in the tissues might lead to the formation of dopamine-derived TIQs produced in the condensation reaction between dopamine and acetaldehyde or 3,4-dihydroxyphenylacetaldehyde[4,5]. Another possible route to the formation of biologically active isoquinolines and β-carbolines is the enzymatic production of formaldehyde from 5-methyltetrahydrofolic acid and subsequent condensation of the formaldehyde with catecholamines or indolamines[6,7]. TIQs are taken up by an active, desmethylimipramine- and cocaine-sensitive mechanism in the nerve endings, and released upon stimulation of the nerves, giving rise to the postsynaptic effect[1].

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For a full account of the effects of TIQs, their cellular localization is important. Catecholamine-derived TIQs are well-known intermediates in a very sensitive fluorescence histochemical method for the cellular localization of catecholamines (CAs), in which CAs are condensed with formaldehyde or glyoxylic acid leading first to the formation of weakly fluorescent TIQs, and subsequently through dehydrogenation (catalyzed by formaldehyde and proteins or acid) to the formation of strongly fluorescent dihydroisoquinolines (DIQs)[8–10]. The uptake and storage of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and 4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline in the adrenergic nerves of the iris has been demonstrated visually both in vitro and in vivo[1,11,12]. The irides, incubated in a solution containing TIQ or obtained from animals injected with TIQ, were exposed to formaldehyde vapour, a procedure which promotes the dehydrogenation

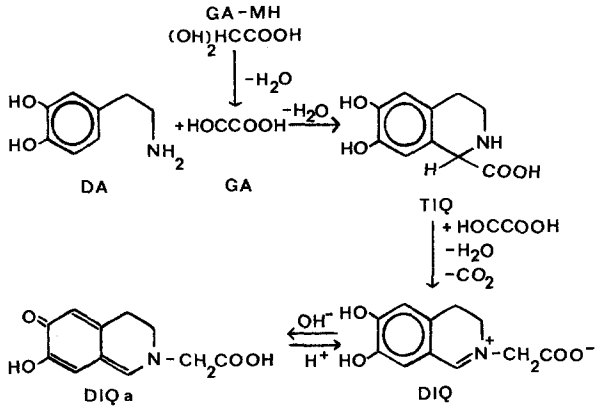
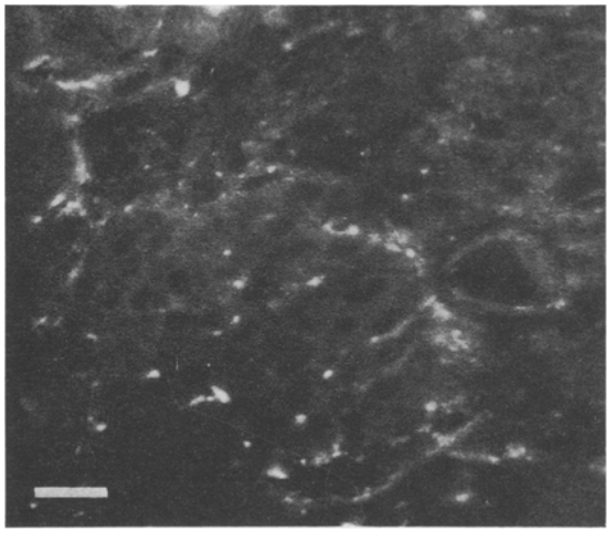


Fig. 1. Proposed reactions of dopamine (DA) with glyoxylic acid (GA) (slightly modified after Lindvall et al.[9]). GA–MH = glyoxylic acid monohydrate, TIQ = 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid, DIQ = 2-carboxymethyl-6,7-dihydroisoquinolinium compound, DIQa = tautomeric quinoidal form.



Figures 2, 3 and 4 are fluorescence photomicrographs of the isoquinoline-injected rat. Fig. 2. The pars intermedia (left side), in which brightly fluorescent nerve endings are seen between nonfluorescent cells and around the lobules. In the pars nervosa (right side) some fluorescent nerves are observed in the zone adjacent to the pars intermedia. No fluorescent nerves are seen around the blood vessel. Calibration bar 25 µm.

of TIQ to strongly fluorescent DIQ. After this treatment, a rich network of fluorescent nerves was visible under fluorescence microscope, providing evidence of the uptake and storage of TIQs. However, endogenous noradrenaline first had to be depleted with reserpine or synthesis inhibitor to avoid interference from its fluorescence. This depletion might well alter the physiological uptake and storage mechanism of the transmitter substance in the nerve endings.

Another possible way to demonstrate the cellular localization of TIQs is by using oxidized forms of TIQs, i.e. DIQs; these can be expected to be demonstrable directly under the fluorescence microscope at low concentrations owing to their strong fluorescence, in the same way as the fluorescence histochemical demonstration of very minute amounts of CAs. It has been estimated, for example, that 5×10^{-4} pg of noradrenaline or dopamine can be detected in a single varicosity of the axon using the formaldehyde-induced fluorescence method and even smaller quantities with glyoxylic acid-induced fluorescence[10]. Using DIQs, the depletion of endogenous CAs is not necessary, provided the treatment of tissue with formaldehyde vapour is omitted. But, on the other hand, it is possible that DIQs and TIQs are handled differently in the tissues. It has, however, been suggested that oxidation of TIQs to DIQs occurs in the nervous system and that DIQs might cause neuronal damage[8], thus making the cellular localization of DIQs important. In this work, some preliminary observations are reported concerning the uptake and storage of DIQ derived from the condensation of dopamine with glyoxylic acid in the hypothalamo-hypophyseal system of the rat.

Materials and methods. Dopamine hydrochloride and glyoxylic acid monohydrate were dissolved at a molar concentration of 1:2 in absolute ethanol and heated in a loosely closed vessel at 70 °C for 3 h in an oven; the vessel was then left open at 70 °C for 17 h and the ethanol allowed to evaporate. The yellow powdery product was scraped off and dissolved in deionized water and the pH adjusted to 7.0 with sodium hydroxide. No dopamine was detected in subsequent thin layer chromatography on silica gel (solvent system: n-butanol-ethanol-acetic acid-water 2:1:1:1) with exposure to formaldehyde vapour[13] or with ninhydrin reagent. Before the above-mentioned reagents were applied, a small spot with very weak blue fluorescence and a larger spot with very strong blue fluorescence were seen in UV light (405 nm, through a Leitz K 470 filter). The weakly fluorescent spot represented 6,7-dihydroxy-1,2,4,4-tetrahydroisoquinoline-1-carboxylic acid and the strongly fluorescent spot a 2-carboxymethyl-6,7-dihydroxy-3,4-dihydro-isoquinolinium compound, which is in equilibrium with its quinoidal form depending on the pH[9,14] (figure 1). 2 slowly moving spots with moderate yellow fluorescence were also detected.

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One of them probably was the ethyl ester of DIQ, and the other the product formed in the reaction of glyoxylic acid with the carboxymethyl side chain of DIQ[14].

The adult male rats were injected i.p. with 50 mg/kg b.wt of synthesized isoquinolines 2.5–3 h before killing by rapid decapitation. The mediobasal hypothalami and the hypophyses of the injected rats and of the control animals were rapidly dissected out, frozen in isopentane precooled in liquid nitrogen and processed through a freeze-drying procedure and embedded in paraffin wax in vacuum (method of Björklund et al.[15]). 7 µm thick sections were deparaffinized with xylene and mounted in Entellan. The sections were examined and photographed with a Leitz Ortholux microscope equipped with an epi-illuminator and HBO 200 high pressure mercury lamp, the primary filters were BG 38, BG 3, TAL 405 (Schott & Gen.) and the secondary filter was a Leitz K 470.

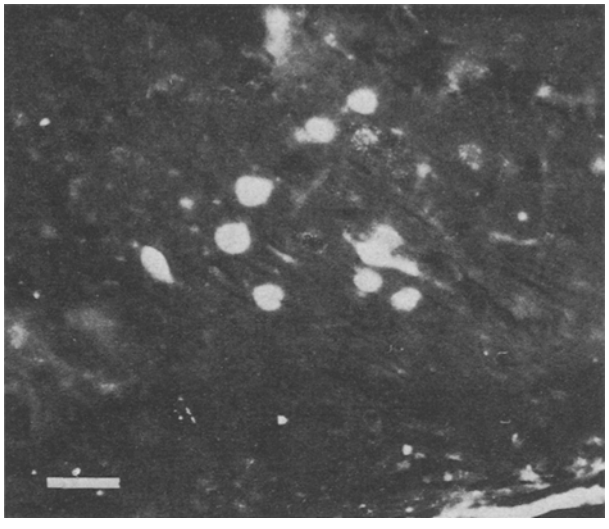

Fig. 3. A frontal section of the nucleus arcuatus, in which many brightly fluorescent neurons are seen scattered against a nonfluorescent background. A corner of the third ventricle is on the left upper part of the picture. Calibration bar 40 µm.

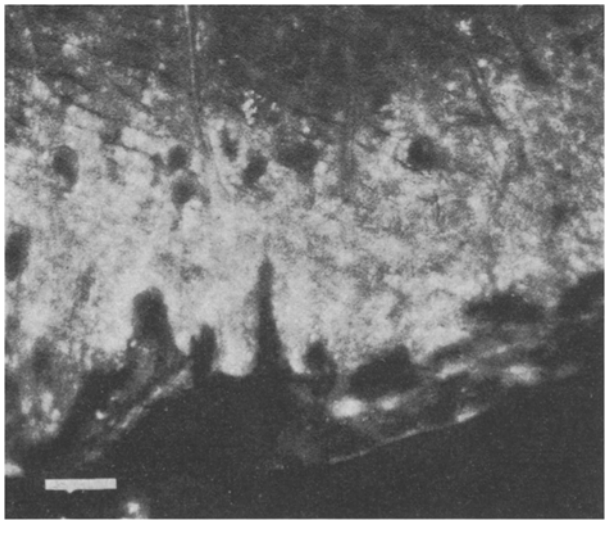

Fig. 4. A frontal section of the median eminence of the hypothalamus. Bright granular fluorescence is seen in the zona externa. Calibration bar 25 µm.

Results and discussion. In the sections obtained from isoquinoline-injected rats, there were some bright blue fluorescent cells in the pars distalis of the hypophysis. In the pars intermedia, blue fluorescent dots and fibres were seen between nonfluorescent cells (figure 2) as well as in the pars nervosa adjacent to the pars intermedia. In the basal hypothalamus, many blue fluorescent neurons were seen in the nucleus arcuatus and periventricularis (figure 3). In the median eminence, a blue fluorescence was seen in the zona externa (figure 4). The fluorescence intensity decreased rapidly in UV light, and was quenched with water. In the sections from the control animals, the only fluorescent structures were orange fluorescent granules in some neurons of the hypothalamus.

It is evident from the results that highly fluorescent DIQ is taken up and stored in some basal hypothalamic neurons, in the nerves of the pars intermedia and in some endocrine cells of the pars distalis of the hypophysis. These hypothalamic neurons are situated anatomically in the same areas as the dopaminergic tuberoinfundibular neuron system[16–18], and it seems that DIQ is taken up by these neurons. The adrenergic nerves of the pars intermedia are of central origin and both dopaminergic and noradrenergic terminals have been detected with microspectrofluorometry[17]. The question remains as to whether DIQ is taken up and stored in dopaminergic or in noradrenergic terminals. No uptake of DIQ in the sympathetic perivascular adrenergic fibres of the hypothalamo-hypophyseal complex was found under the experimental conditions used in the present study. The uptake and storage of DIQ in the cells of the adenohypophysis seem to occur in the same cells which exhibit formaldehyde-induced fluorescence after the injection of L-dopa or 5-HTP, a phenomenon whose physiological significance is not known[19]. It is also interesting that, after administering large doses of L-tryptophan to the rats, blue fluorescent cells were observed in the pars distalis without formaldehyde vapour treatment[20]. It has been suggested that the fluorescence is derived from some fluorescent metabolite of tryptophan. It is possible that the fluorescence was caused by fluorescent dihydro-β-carboline formed by the in vivo condensation of tryptophan or tryptamine with aldehyde leading first to tetrahydro-β-carboline and then through autoxidation to dihydrocarboline analogously with the formation of TIQs from dopamine and acetaldehyde after the administration of L-dopa[4].

The results of the present study show that exogenously administered DIQs can be detected at the cellular level with fluorescence histochemistry. The same technique is perhaps useful for the demonstration of dihydro-β-carbolines, condensation products of indolamines and aldehydes. DIQs formed in vivo from their immediate precursors, TIQs, can also be expected to be demonstrable on the same principle, if this chemical reaction does take place. In the nervous tissue, the uptake and storage of DIQs seems to be a neuronal process, and it is possible that TIQs are handled in the same way.

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