Short communications

In vitro effect of 6-hydroxydopamine on isolated rat atria

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In vitro treatment with 6-hydroxydopamine (6-OHDA), but not with oxidized 6-hydroxydopamine, caused transient, positive inotropic and chronotropic responses in the isolated rat atria. In the presence of rat blood plasma, the excitatory effect of 6-hydroxydopamine persisted unless the drug was washed out by fresh medium. 6-Hydroxydopamine failed to elicit the excitative response in the atria obtained from reserpinized rats or rats treated with 6-hydroxydopamine *in vivo*. *In vitro* treatment with 6-hydroxydopamine did not cause the development of supersensitivity to noradrenaline. Cocaine and desipramine, but not bretylium, inhibited the excitative action of 6-hydroxydopamine. No tachyphylaxis developed after repeated exposure to 6-hydroxydopamine *in vitro*. Propranolol, but not phentolamine, blocked the excitatory effect of 6-hydroxydopamine. It is thus concluded that *in vitro* treatment with 6-hydroxydopamine may cause atrial stimulation by an indirect action involving the release of catecholamines as a result of its displacement at the nerve ending.

Morphological studies indicated that 6-hydroxydopamine (6-OHDA) is a unique chemical agent in that it can selectively cause a complete deterioration of adrenergic nerve terminals when injected systemically (Tranzer & Thoenen, 1967, 1968; Knyihar, Ristovsky, Kalman & Csillik, 1969; Bennett, Burnstock, Cobb & Malmfors, 1970). Recently it was reported that local perfusion of the sinus node with 6-hydroxydopamine produces a positive chronotropic action and a marked noradrenaline depletion of the perfused region compatible with a localized degeneration of the sympathetic nerve terminals (Elharrar, de Champlain & Nadeau, 1971). On the other hand, no reports have appeared concerning the effect of the *in vitro* application of 6-hydroxydopamine on the isolated tissue.

The present experiments, therefore, were undertaken to clarify whether the *in vitro* application of 6-hydroxydopamine can displace catecholamine and cause degeneration of the adrenergic nerve terminal in this isolated tissue. In these preliminary experiments the action of 6-hydroxydopamine on isolated rat atria was studied.

Methods.—Hearts were isolated from Wistar rats (weight, 200 g; both sexes) which had been stunned by a blow on the head and bled via the carotid arteries. The atria dissected from the hearts were suspended in 50 ml tissue baths containing Krebs-Ringer bicarbonate medium of the following composition (mM): Na^+, 145; K^+, 6·02; Ca^{++}, 1·22; Mg^{++}, 1·33; Cl^-, 126; HCO_3^-, 25·3; PO_4^{--}, 1·2; SO_4^{--}, 1·33; glucose, 5·5; all in distilled, deionized water. The solution was maintained at 37° C and equilibrated before and during the experiment with a gas mixture of 95% O_2 and 5% CO_2 (pH 7·4) in the tissue bath. One end of the tissue was tied to an anchoring glass rod in the bath solution, and the other end was attached to a transducer by means of a thread. After applying 750 mg tension, the atria were allowed to equilibrate for 60–90 min; this loaded tension was maintained throughout the experiment. The preparation was washed repeatedly and allowed to recover for at least 20 min before adding the next agent. Spontaneous rate and force of contraction of the atria were measured as beats per minute and tension in milligrammes, respectively. These atrial activities were recorded by a strain-gauge transducer (Grass FT.03) and a Grass polygraph.

In some experiments, atria were obtained from rats that had been injected intraperitoneally with reserpine (4 mg/kg) or intravenously with 6-hydroxydopamine (75 mg/kg) 24 h before being killed. The 6-hydroxydopamine dissolved in distilled, deionized water containing 2 mg/ml of ascorbic acid immediately before use. The following agents were used: 6-hydroxydopamine hydrobromide, (−)-noradrenaline bitartrate, desipramine (desmethylimipramine), bretylium tosylate, propranolol hydrochloride (Inderal), phentolamine mesylate and reserpine (Serpasil, CIBA). The plasma was prepared from blood of untreated rats using a clinical centrifuge. The concentrations of agents used are ex-

pressed as the final concentration in the tissue bath.

Results.—Treatment with 6-hydroxydopamine (10^{-4} and 10^{-5}M) *in vitro* caused transient, positive inotropic and chronotropic responses in the isolated rat atria. The excitatory effect of 6-hydroxydopamine disappeared about 10 min after its application. When oxidized 6-hydroxydopamine (10^{-4}M ; using 0·1 N NaOH) was applied to the atria, it had no excitatory effect on the developed tension and contraction rate of the atria. On the other hand, in the presence of medium containing blood plasma (2%), the positive inotropic and chronotropic effects produced by 6-hydroxydopamine (10^{-4}M) persisted for prolonged periods unless the drug was washed out by fresh medium.

In atria obtained from animals injected with 6-hydroxydopamine, *in vitro* treatment with 6-hydroxydopamine (10^{-4}M) failed to elicit any excitative response. A similar absence of the excitatory response to 6-hydroxydopamine (10^{-4}M) was observed in the atria obtained from reserpinized animals.

When the positive inotropic and chronotropic effects produced by treatment with 6-hydroxydopamine (10^{-5}M) had returned to the initial control magnitude, a second application of 6-hydroxydopamine (10^{-5}M) produced responses similar to those caused by the first application. Furthermore, even after five successive applications of 6-hydroxydopamine (10^{-5}M), no tachyphylaxis developed to 6-hydroxydopamine. The positive inotropic and chronotropic response to noradrenaline (10^{-10} to 10^{-6}M) was not modified after *in vitro* treatment with 6-hydroxydopamine (10^{-5}M) for 10 minutes.

In untreated atria and even in atria treated with 6-hydroxydopamine (10^{-5}M) for 20 min, nicotine and tyramine (both 10^{-5}M) have similar inotropic and chronotropic effects. Also in untreated atria and atria treated with nicotine or tyramine (both 10^{-5}M) for 20 min, 6-hydroxydopamine (10^{-5}M) elicited similar responses.

Pretreatment for 20 min with cocaine or desipramine (both 10^{-5}M), blocked the stimulant action of 6-hydroxydopamine (10^{-5}M) on the developed tension and contraction rate of the atria. On the other hand, after treatment with bretylium (10^{-5}M) for 20 min, the positive inotropic and chronotropic effects produced by 6-hydroxydopamine (10^{-5}M) were not modified. Furthermore, propranolol (10^{-6} M), but not phentolamine (10^{-6}M), inhibited the positive inotropic and chronotropic action of 6-hydroxydopamine (10^{-4}M).

Discussion. — 6-Hydroxydopamine is rapidly oxidized in a neutral aqueous solution (Tranzer & Thoenen, 1968) and the oxidized form of 6-hydroxydopamine is ineffective on the nerve element of isolated mice atria and irides in *in vitro* experiments (Jonsson & Sachs, 1970). Thus, the short acting character of 6-hydroxydopamine in Ringer medium with an exogenous oxygen supply was probably due to its rapid oxidative destruction. The persistence of the 6-hydroxydopamine effect in the presence of plasma may be related to the inhibition of the otherwise rapid oxidative destruction which occurs in this medium, since only the non-oxidized form is active on the sympathetic neuronal site. Therefore, blood plasma may have the ability to reduce oxidation.

The absence of an excitatory response to 6-hydroxydopamine (10^{-4}M) after pretreatment with either reserpine or 6-hydroxydopamine *in vivo* suggests that a certain amount of tissue catecholamine may be required for such responses.

The fact that tachyphylaxis did not develop eliminates the possibility that the brief stimulant action of 6-hydroxydopamine is due to the exhaustion of releasable catecholamines from nerve endings. Treatment with 6-hydroxydopamine *in vivo* caused development of atrial supersensitivity to noradrenaline (Brus, Hess & Jacobowitz, 1970 ; Nadeau, de Champlain & Tremblay, 1971 ; Shibata, Kuchii & Kurahashi, unpublished). However, atrial response to noradrenaline (10^{-10} to 10^{-6}M) was not modified after *in vitro* treatment with 6-hydroxydopamine (10^{-5}M) for 10 minutes. This suggests that the excitatory effect of 6-hydroxydopamine may be due to an indirect action involving the liberation of noradrenaline from nerve endings as a result of the displacement of noradrenaline by 6-hydroxydopamine as suggested by Bartholini, Richards & Pletscher (1970) in the central nervous system. Nicotine and tyramine (both 10^{-5}M), which act through catecholamine-release mechanisms caused similar inotropic and chronotropic effects, both in untreated and 6-hydroxydopamine (10^{-5} M) treated atria. The absence of cross-

tachyphylaxis to the subsequent application of nicotine or tyramine suggests that the amount of catecholamine released by 6-hydroxydopamine is much less than that released by tyramine or nicotine, and the short duration of the response to 6-hydroxydopamine is consistent with this concept. Alternatively, 6-hydroxydopamine may release catecholamine from sites other than those affected by nicotine or tyramine.

Cocaine and desipramine, but not bretylium blocked the stimulant action of 6-hydroxydopamine (10^{-5}M). Since 6-hydroxydopamine is taken up and stored by peripheral adrenergic neurones (Johnsson & Sachs, 1970), this block of the stimulant action of 6-hydroxydopamine may be attributed to the inhibition of uptake of 6-hydroxydopamine by cocaine and desipramine and a consequent lack of noradrenaline release from nerve endings. The absence of the inhibitory action of bretylium, on the release of catecholamines, suggests that the catecholamines may have been displaced from the axoplasm of the nerve terminals by the 6-hydroxydopamine. The inhibition of positive inotropic and chronotropic actions of 6-hydroxydopamine by propranolol, but not by phentolamine suggests a role of beta-adrenoceptors in such action.

These results lead us to conclude that the *in vitro* treatment with 6-hydroxydopamine may cause atrial stimulant effects by an indirect action involving the release of catecholamines by displacement from nerve endings.

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