Tyramine-like Effect of Cyclocytidine (2,2′-Anhydro-1-beta-Arabinofuranosylcytosine Hydrochloride), an Antineoplastic Agent

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OGURO, K. and HASHIMOTO, K. Tyramine-like Effect of Cyclocytidine (2,2′-Anhydro-1-beta-Arabinofuranosylcytosine Hydrochloride), an Antineoplastic Agent. Tohoku J. exp. Med., 1978, 124 (1), 83-90 — Since cyclocytidine (2,2′-anhydro-1-beta-arabinofuranosylcytosine hydrochloride) was introduced as an antineoplastic agent for the treatment of lymphatic leukemia, sinus acceleration and an increase in systemic blood pressure has been reported as its systemic effects in the clinical cases. These cardiovascular effects of cyclocytidine were observed also in anesthetized dogs, but not in reserpine-pretreated animals. Increases in heart rate and in systemic blood pressure were prevented by propranolol and phenolamine, respectively. The mechanism of these sympathomimetic effects was further analysed in the excised, blood-perfused canine sinoatrial node and papillary muscle preparations with a support dog. Positive chronotropic and inotropic responses to cyclocytidine were abolished by desipramine, propranolol, and pretreatment with reserpine but not by tetrodotoxin and hexamethonium. The tyramine-like actions of cyclocytidine at adrenergic neuronal terminals were discussed in conjunction with the uptake mechanism of the drug into the tumor cells. — cyclocytidine; tyramine-like sympathomimetic effect; positive chronotropic and inotropic effects; blood-perfused preparations of canine sinoatrial node and papillary muscle

A newly synthetized 2,2′-anhydro-1-beta-arabinofuranosylcytosine hydrochloride (cyclocytidine, Fig. 1., Kanai et al. 1970; Kikugawa and Ichino 1970) was introduced as an antitumor agent against a variety of experimental animal tumors and acute leukemias of man (Hoshi et al. 1971, 1972; Nakahara and Tokuzen 1972; Venditti et al. 1972; Sakai et al. 1972, 1976; Kimura 1973; Sakano 1974; Fujioka et al. 1976; Yamada and Kimura 1975; Ise et al. 1976). Concerning the mode of antineoplastic effect, cyclocytidine was found to be transformed to aracytidine (1-beta-arabinofuranosylcytosine) in the tissue cell which inhibits both DNA polymerase and RNA reductase (Chu and Fisher 1962; Furth and Cohen 1968; Inagaki et al. 1969; Graham and Whitmore 1970a, b; Hoshi et al. 1973a, b). A positive chronotropic effect of cyclocytidine was observed in conscious beagle dogs with therapeutic doses, while aracytidine had no cardiodynamic effect at all (Hirayama et al. 1972).

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In the present experiments, these effects of cyclocytidine were further analysed in isolated preparations of the canine atrium and the papillary muscle which were perfused with arterial blood of a support dog.

**MATERIALS AND METHODS**

Thirty-two dogs of either sex, weighing 8 to 18 kg, were anesthetized with 30 mg/kg of sodium pentobarbital given i.v. A tracheal tube was inserted and animals were respired artificially with room air with a respiration pump (Harvard Appartus Model 607). Femoral artery blood pressure was measured by a transducer (San-ei Sokki Type 1206 B). The heart rate was recorded by a cardiotachometer which was triggered by R waves of lead II of the electrocardiograph.

Two animals in the first group of the experiments were prepared for measuring coronary blood flow. After i.v. administration of sodium heparin, 500 U/kg, the chest was opened by the midsternal incision and the Morawitz cannula was inserted into the coronary sinus. Flow rate of the coronary sinus was measured with an electromagnetic flowmeter (Nihon Kohden MF-2). Three animals in the second group were pithed and adrenalectomized by transection of the spinal cord at C 1 with suprasegmental destruction after vagotomy and followed by bilateral adrenalectomy. Three animals in the third group were reserpine-pretreated and pithed. Animals were treated with 1 mg/kg of reserpine given s.c. for 2 days prior to the experiment.

The excised sinoatrial node and the excised papillary muscle preparations were cross-circulated with the arterial blood of a support dog. After i.v. administration of sodium heparin, 500 U/kg, the heart of the dog anesthetized with sodium pentobarbital was excised and immediately plunged into cold Tyrode’s solution at about 4°C equilibrated with a gas mixture consisting of 95% O₂ and 5% CO₂.

Five sinoatrial node preparations were arranged as follows: The sinus node artery was cannulated at its origin in the right coronary artery. The excised right atrium was placed in a glass container with a double wall glass jacket which was kept at 38°C by circulating warm water, and cross-circulated at a constant perfusion pressure of 100 mmHg with arterial blood of a support dog by the aid of a peristaltic pump (Tokyo Rika Kikai, Co., Ltd. C-16). The sinus rate was recorded by a cardiotachometer triggered by the right atrial electrogram. The details of the preparation were described in a previous paper (Kubota and Hashimoto 1973).

Five papillary muscle preparations were arranged as follows: The anterior septal artery was dissected and cannulated. All branches of this artery except those to the anterior papillary muscle were ligated, and the unirrigated part of the muscle was removed. The papillary muscle was placed in another glass container and cross-circulated as described above. The tendinous end of the papillary muscle was connected to a force-displacement transducer (Nihon Kohden SB-1T) by a fine silk thread and the muscle loaded with a weight of 1.5 g was driven at a constant frequency of 2 Hz with a rectangular pulse of 2 volts and 5 msec duration by an electronic stimulator (Nihon Kohden MSE-3) through a bipolar electrode in contact with the base of the muscle. The preparation of the blood-perfused papillary muscle has been described in detail by Endoh and Hashimoto (1970).

Drug solution was injected either intra-arterially into the sinus node artery and the anterior septal artery or intra-venously to the support dog. In two experiments, the excised sinoatrial node and the excised papillary muscle were simultaneously perfused with arterial blood of a support dog.

Drugs used were cyclocytidine hydrochloride (Kohjin), aracytidine hydrochloride (Kohjin), dl-propranolol hydrochloride (Sumitomo Kagaku), phenolamine (Ciba Geigy), l-isoprenaline hydrochloride (Nakarai), l-noradrenaline (E. Merck), tyramine hydrochloride (Nakarai), desipramine (desmethylimipramine, Ciba Geigy), reserpine (Nakarai) and sodium heparin (Sigma). Drugs were diluted with 0.9% saline solution in each experiment.
RESULTS

Effects of cyclocytidine and aracytidine on the systemic blood pressure, heart rate and electrocardiogram

Cyclocytidine, 1 to 30 mg/kg i.v., caused a dose-dependent increase in the systemic blood pressure and the heart rate as shown in Fig. 2. The pulse pressure and the coronary sinus outflow also increased, and ECG showed sinus tachycardia. Sustained increases in these parameters induced by 30 mg/kg i.v. of cyclocytidine were abolished by propranolol, 0.5 mg/kg i.v., except an increase in systemic blood pressure. Increases in systemic blood pressure and in heart rate were observed in normal and pithed dogs even after adrenalectomy: these effects were completely blocked by alpha and beta adrenergic blocking agents (Fig. 3). These responses

![Chemical structures of cyclocytidine and aracytidine](image)

Cyclocytidine
\[\text{C}_{9}\text{H}_{12}\text{N}_{2}\text{O}_{4}\cdot\text{HCl}\]
Molecular weight 261.67

Aracytidine
\[\text{C}_{9}\text{H}_{12}\text{N}_{2}\text{O}_{4}\cdot\text{HCl}\]
Molecular weight 278.72

Fig. 1. Chemical structures of 2,2'-anhydro-1-beta-D-arabinofuranosylcytosine hydrochloride (cyclocytidine hydrochloride) and 1-beta-D-arabinofuranosylcytosine hydrochloride (aracytidine hydrochloride).

![Graph showing effects on blood pressure, heart rate, coronary sinus outflow, and ECG](image)

Fig. 2. Effect of the i.v. administration of cyclocytidine and aracytidine on the systemic blood pressure (SBP), heart rate (HR), coronary sinus outflow (CBF) and electrocardiogram (ECG). NA, noradrenaline.
Fig. 3. Effect of the i.v. administration of cyclocytidine (Cycloc.) and aracytidine (Arac.) on the systemic blood pressure (SBP) and heart rate (HR) in anesthetized, pithed, adrenalectomized, and reserpinized dogs. Treatments with propranolol, phentolamine and reserpine abolished both sinus acceleration and hypertension. NA, noradrenaline.

were not induced in reserpinized and pithed animals. Aracytidine even in a large dose of 100 mg i.v. had almost no effect.

Simultaneous observation of the cardiovascular effects of cyclocytidine on the excised and blood-perfused sinoatrial node and papillary muscle preparations and the support dog

An intravenous administration of cyclocytidine and noradrenaline (NA) to the support dog caused a sustained rise in the systemic blood pressure and an increase in heart rate with an increased pulse pressure in a dose-dependent manner. The excised sinoatrial node and the papillary muscle preparations responded to these drugs with sustained positive chronotropic and inotropic effects, respectively, when sinus tachycardia and an increase in systemic blood pressure were induced in the support dog (Fig. 4).

Observations on the excised, blood-perfused sinoatrial node preparation with a support dog

Cyclocytidine caused a positive chronotropic effect in a dose-dependent manner. The positive chronotropic effect of cyclocytidine was compared with those induced by NA and tyramine (Fig. 5). The positive chronotropic responses to cyclocytidine and tyramine were long lasting compared with that to NA, and cyclocytidine was approximately 300 times less potent than tyramine on a weight basis. Tetrodotoxin (TTX) did not block the effects of cyclocytidine and NA, but
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Fig. 4. Effect of the i.v. administration of cyclocytidine on the systemic blood pressure and heart rate of a support (donor) dog and those on the excised, blood-perfused atrium and papillary muscle preparations which were cross-circulated with arterial blood of a support dog.

SBP, systemic blood pressure; HR, heart rate (beats/min); SAR, sino-atrial rate; PMT, tension of the papillary muscle; SA, sinus node; PM, papillary muscle; and NA, noradrenaline.

Fig. 5. Effect of treatments with tetrodotoxin (TTX), propranolol, desmethylimipramine and reserpine on the cyclocytidine-induced positive chronotropic response to the excised, blood-perfused canine atrium preparation with a support (donor) dog. SAR, sino-atrial rate (beats/min); NA, noradrenaline; and Tyr, tyramine.

propranolol inhibited them markedly. Desipramine infused at a rate of 10 µg/min completely abolished the effects of tyramine and cyclocytidine but the response to NA was rather intensified, and a dose-dependent effect of cyclocytidine was absent in the atrial preparation excised from the reserpinized dog as shown in the lower trace of Fig. 5. Aracytidine, 3 mg i.a., had no direct effect on the sinus node.
Observations on the excised, blood-perfused papillary muscle preparation with a support dog

Both cyclocytidine and aracytidine caused a positive inotropic effect. The positive inotropic responses to these compounds were compared with those to NA and tyramine (Fig. 6). Cyclocytidine was approximately 100 times less potent than tyramine on a weight basis. Hexamethonium (C₆) and TTX did not modify the responses to cyclocytidine and NA, but propranolol completely abolished those responses except that to aracytidine. Desipramine, 10 μg/min i.a., abolished the positive responses to both tyramine and cyclocytidine but not that to NA. The positive inotropic effect of cyclocytidine was hardly observed in reserpine-pretreated preparation even with a large dose. Although aracytidine induced a slight inotropic effect, this was not modified by C₆, TTX, propranolol, or reserpine-pretreatment.

Fig. 6. Effect of treatments with hexamethonium, tetrodotoxin (TTX), propranolol, desmethylimipramine and reserpine on the cyclocytidine- and aracytidine-induced positive inotropic responses to the excised, blood-perfused papillary muscle preparations. PMT, tension of the papillary muscle; NA, noradrenaline; and Tyr, tyramine.

Discussion

Hirayama et al. (1972) observed a sinus acceleration induced by an i.v. administration of cyclocytidine in conscious beagle dogs. In these experiments, the excised, blood-perfused heart preparations by use of support dog have definite merits to observe simultaneously direct and indirect effects of drugs on the pace-maker activity of the sino-atrial node and on the contractile force of the ventricular muscle.

An intravenous administration of more than 3 mg/kg of cyclocytidine caused sympathomimetic effects on the cardiovascular system in a dose-dependent manner not only in the whole animal but also in the pithed and adrenalectomized ones. An increase in systemic blood pressure was abolished by treatment of phentolamine. Positive chronotropic and inotropic effects were observed both in vivo and in vitro.
preparations which were blocked by propranolol and desipramine, and were absent in reserpine-pretreated animals. These effects, however, were not diminished by treatment with C₈ and TTX. Thus, direct effect on the myocardial tissue, nicotinic action to the sympathetic ganglia and adrenal glands can be excluded. Through these results we can conclude that cyclocytidine releases catecholamine from the sympathetic nerve terminals. The pharmacological feature of releasing action of catecholamine by cyclocytidine was similar to that of tyramine, because both were blocked by an infusion of desipramine.

Supposedly the mode of action of tyramine or tyramine type compounds has been thought to be the displacement of endogenous neurotransmitter from the vesicles of sympathetic nerve terminals, which is defined as an indirect sympathomimetic effect (Iversen 1967).

It is worthy to note that sympathomimetic effects induced by an antitumor agent such as cyclocytidine are blocked by treatment of desipramine as similar to tyramine. Cyclocytidine has been reported to be readily transferred through the cell membrane and then transformed to aracytidine inside the cell as mentioned above. Furthermore, aracytidine never showed any tyramine-like action. Thus, two questions arise; the first one is whether the common event exists between the transport mechanism of cyclocytidine through the cell membrane of tumor cell and the uptake mechanism at the sympathetic nerve terminals, and the second one is whether cyclocytidine itself or aracytidine biotransformed from cyclocytidine inside the cell acts as an actual releaser of endogenous neurotransmitter from vesicles of the sympathetic nerve terminals. It must be examined whether desipramine will modify the effectiveness of cyclocytidine as an antitumor agent.

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References


