Biochemical Pharmacology, Vol. 35, No. 12, pp. 2047–2049, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

Inhibition by hydralazine of catecholamine biosynthesis in cultured bovine adrenal medullary cells

(*Received* 12 *August* 1985; *accepted* 6 *December* 1985)

Hydralazine is widely used for the treatment of hypertension, but the mechanism of the hypotensive action of this drug has not been elucidated completely. Hydralazine may lower blood pressure as a result of the dilation of blood vessels, and the vasodilating action of this drug may be due to direct action on vascular smooth muscle [1–3]. On the other hand, hydralazine has been shown to reduce the tension of blood vessels through a modulation of neurotransmitter release from sympathetic nerves [4–7]. Furthermore, the administration of hydralazine has been reported to cause an inhibitory effect on the catecholamine-synthesizing enzymes in brain and sympathetic nerves [8–11]. It has been proposed, therefore, that hydralazine may indirectly cause the dilating action on blood vessels as a result of diminished sympathetic nerve activity produced by an alteration in the biosynthesis as well as the release of neutrotransmitters.

In our previous studies, the direct action of hydralazine on tyrosine hydroxylase (TH, EC 1.14.16.2) and dopamine β-hydroxylase (DBH, EC 1.14.17.1), the catecholamine-synthesizing enzymes, was investigated, and hydralazine was found to cause an irreversible inhibition of both enzymes [12]. In the present work, therefore, we studied the effect of hydralazine on catecholamine (CA) biosynthesis in cultured bovine adrenal medullary cells and found that hydralazine caused a decrease in CA biosynthesis as a result of inhibition of both TH and DBH within the intact cell.

Materials and methods

Bovine adrenal medullary cells were enzymatically dispersed according to the method described by Schneider *et al.* [13]. Cells were plated on a 60-mm diameter culture dish at a density of 6×10^6 cells and then cultured for 3 days in 4 ml of Eagle's Minimum Essential Medium (MEM) containing 10% heat-inactivated calf serum, 2 mM glutamine, penicillin (100 units/ml), streptomycin (100 μg/ml) and fungizone (0.3 μg/ml). After removing the culture medium, plated cells were washed with Krebs–Ringer phosphate buffer (KRPB), pH 7.4, and incubated with 20 μM [^{14}C]tyrosine (0.25 μCi/ml) or [^{14}C]DOPA (0.25 μCi/ml) at 37° for 30 min in 2 ml of KRPB containing 0.5% bovine serum albumin (BSA). At the end of the incubation, the reaction medium was discarded quickly, and the cells were lysed by adding 2 ml of 0.4 N perchloric acid and freeze-thawing. The acid extract was applied on an ion-exchange column (Duolite C-25, 0.4 × 7 cm), and the fractions of radioactive CA formed from ^{14}C-labeled substrates were determined according to the method reported by Itoh *et al.* [14].

In our preliminary experiments, the fractions obtained from the ion-exchange column were analyzed by high performance liquid chromatography (HPLC). We found that almost all radioactivity was recovered in the fractions corresponding to dopamine (DA) and norepinephrine (NE). The formation of radioactive epinephrine from ^{14}C-labeled substrates was not observed under the experimental conditions used here.

Student's *t*-test was used to determine statistical significance.

Hydralazine hydrochloride (Apresoline) was donated by the Kyowa Fermentation Industry Co. (Tokyo, Japan). L-[U-^{14}C]Tyrosine and L-[3-^{14}C]DOPA were purchased from the Amersham Corp. Collagenase (Type I), bovine serum albumin (Fraction V) and trypsin inhibitor (soybean) were obtained from the Sigma Chemical Co.

Results and discussion

The effect of hydralazine on CA biosynthesis was studied using cultured bovine adrenal medullary cells. As shown in Fig. 1, the formation of radioactive CA from [^{14}C]tyrosine

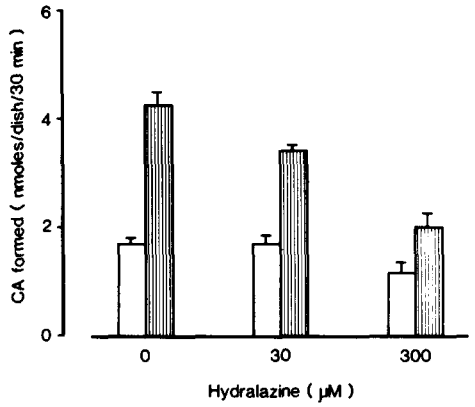

Fig. 1. Effect of hydralazine on radioactive CA biosynthesis from [^{14}C]tyrosine in cultured adrenal medullary cells. Plated cells were incubated with 20 μM L-[U-^{14}C]tyrosine at 37° for 30 min in the presence and absence of 100 μM carbamylcholine. The basal (open column) and carbamylcholine-stimulated (striped column) formation of radioactive amines was determined by the method described in the text. Values are the mean ± S.E. of six experiments. A significant decrease in the basal CA formation was obtained by 300 μM drug ($P < 0.05$), and the significant inhibition of stimulated CA formation by hydralazine was observed at 30 μM ($P < 0.01$) and 300 μM ($P < 0.005$).

was reduced slightly but significantly by 300 μM hydralazine. The stimulation of the cells by carbamylcholine resulted in an approximate 2.5-fold increase in CA formation, and the inhibitory effect of hydralazine on the formation of CA was observed in these stimulated cells. The stimulated CA formation was inhibited significantly by 30 μM hydralazine and 50% inhibition was obtained by 300 μM drug. These results seem to indicate that hydralazine may decrease the formation of radioactive CA from [^{14}C]tyrosine as a result of the inhibition of TH. Furthermore, to investigate the effect of hydralazine on the conversion of DA to NE, radioactive DA and NE formed from [^{14}C]tyrosine were determined separately. Hydralazine was shown to decrease the formation of radioactive DA (Fig. 2A) and NE (Fig. 2B) from [^{14}C]tyrosine. The decrease in NE formation was found to be more profound than that in DA formation under these conditions. The concentration of hydralazine that caused 50% inhibition of

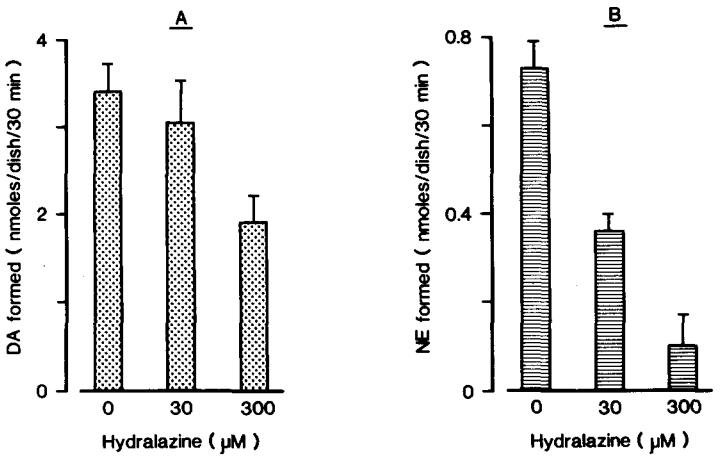

Fig. 2. Inhibitory effect of hydralazine on radioactive DA (A) and NE (B) formation from [^{14}C]tyrosine in cultured adrenal medullary cells. Plated cells were incubated with 20 μM L-[U-^{14}C]tyrosine at 37° for 30 min in the presence of 100 μM carbamylcholine, and radioactive DA and NE formed during the reaction were separated and measured as described in the text. Values are the mean ±S.E. of six experiments. A significant decrease in the DA formation was obtained by 30 μM ($P < 0.1$) and 300 μM hydralazine ($P < 0.005$), and the NE formation was inhibited significantly by 30 and 300 μM drug ($P < 0.005$).

DA formation inhibited NE formation more than 80%. These results suggest the possibility that the decrease in NE formation caused by hydralazine may not be simply due to a decrease in the concentration of DA within the cell but, probably, to an inhibitory effect on the conversion of DA to NE. To confirm this possibility, the effect of hydralazine on the formation of radioactive NE was investigated using [^{14}C]DOPA instead of [^{14}C]tyrosine as a substrate. Although the formation of radioactive DA from [^{14}C]DOPA was not affected by hydralazine (Fig. 3A), the inhibitory action of this drug on radioactive NE formation was observed under the same conditions (Fig. 3B), and the potency of the inhibitory action was found to be nearly identical to that observed in the experiment using [^{14}C]-tyrosine (Fig. 2B). In addition, the inhibitory action of hydralazine was also observed in the absence of carbamylcholine (data not included in Figs. 2 and 3). These findings therefore seem to indicate that hydralazine may be able to inhibit the conversion of DA to NE as well as the hydroxylation of tyrosine to DOPA in cultured bovine adrenal medullary cells. The inhibition of tyrosine hydroxylation seems to indicate an inhibitory action of hydralazine on TH itself. In contrast, it is still unclear whether or not the inhibition of the conversion of DA to NE may directly reflect the inhibitory action of this drug on DBH itself. It is known that the transport of DA into chromaffin granules is a prerequisite for the hydroxylation of DA to NE, and this process is therefore considered to be an important mechanism for the regulation of NE biosynthesis *in vivo* [15, 16]. Recently, it has been shown that the cellular concentration of ascorbic acid may be involved in the regulation of the conversion of DA to NE providing reduc-

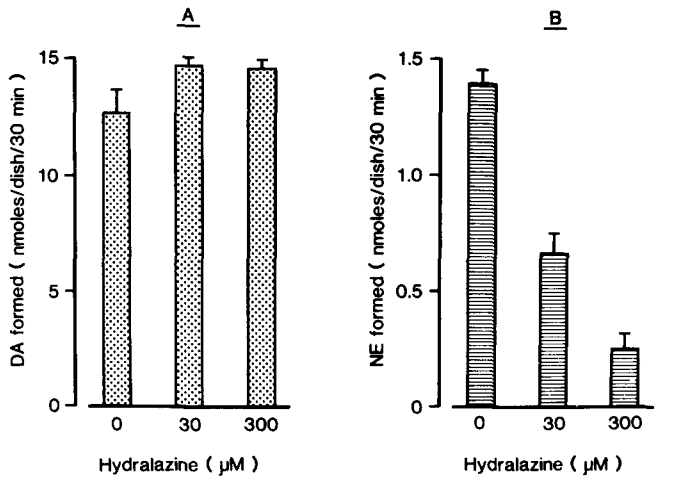

Fig. 3. Inhibitory effect of hydralazine on radioactive NE formation from [^{14}C]DOPA in cultured adrenal medullary cells. Plated cells were incubated with 20 μM L-[3-^{14}C]DOPA at 37° for 30 min in the presence of 100 μM carbamylcholine, and radioactive DA (A) and NE (B) formed during the reaction were isolated and determined as described in the text. Values are the mean ± S.E. of four experiments. A significant change in the DA formation was not observed, but the NE formation was inhibited significantly by 30 and 300 μM hydralazine ($P < 0.005$).

ing equivalents for the enzymatic reaction in cultured bovine adrenal medullary cells [17, 18]. In view of these facts, it seems possible that the inhibitory effect of hydralazine on the formation of NE may be due to inhibition of either the transport of DA into granules or the supplementation of reducing equivalents for the hydroxylation cycle. However, the 50% inhibitory concentration (IC_{50}) of hydralazine calculated from the results in Figs. 2 and 3 was found to be approximately 300 μM for the hydroxylation of tyrosine (Fig. 2A) and 30 μM for the conversion of DA to NE (Figs. 2B and 3B); these values were shown to be almost similar to the IC_{50} for TH and DBH (approximately 100 and 20 μM respectively) obtained from our previous experiments using the isolated enzymes [12]. This fact, therefore, seems to indicate that the inhibitory effect of hydralazine on the formation of both radioactive DA and NE from [^{14}C]tyrosine may be due to inhibition of TH and DBH, the rate-limiting enzymes in CA biosynthesis, by this drug within the intact cell.

In summary, the present studies show that hydralazine caused an inhibitory effect on basal and stimulated CA biosynthesis in cultured bovine adrenal medullary cells and also suggest that this drug may be able to suppress the hydroxylation of tyrosine to DOPA and the conversion of DA to NE as a result of the direct inhibition of both TH and DBH *in vivo*.

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Biochemical Pharmacology, Vol. 35, No. 12, pp. 2049–2051, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

Transducing signals involved in the activation of resting tonsillar B cells*

(*Received* 29 *November* 1985; *accepted* 9 *January* 1986)

Purified G0 human B cells provide a good system for investigating the biochemistry of signal transduction pathways involved in their activation and proliferation. Resting B cells can be stimulated to proliferate by a number of different agents which include complete mitogens, such as SAC (*Staphyloccus auerus* Cowan strain 1), immobilised anti-immunoglobulin and EBV, and incomplete mitogens such as $F(ab)_2$ fragments of anti-immunoglobulin which require the presence of T cell derived products such as B-cell growth factor (BCGF) to complete the cell cycle. Polyclonal mitogens have been shown to stimulate the turnover of phosphatidylinositol lipids in lymphocytes (reviewed in [1]). Recently, several workers have shown that hydrolysis of phosphatidylinositol 4,5 bis-phosphate (PI 4,5-P2) occurs in T cell derived lines [2, 3] or in purified T cells and B cells when surface receptors are crosslinked by polyclonal mitogens [4–6]. A general role for inositol phospholipid metabolism in the control of cell growth is supported by the observation that binding of the mitogen platelet derived growth factor (PDGF) to 3T3 cells results in the breakdown of PI 4,5-P2 [7]. Whether this hydrolysis is an invariant corollary of B cell mitogenesis and the analysis of the signalling processes achieved by the component arms of this hydrolytic pathway are the focus of this investigation. Analysis of the various stages of B cell activation was undertaken using monoclonal antibodies to cell surface antigens which identify various putative receptors.

* Part of this work was presented at the 3rd Conference of the European Society for Biochemical Pharmacology "Phorbol esters, protein kinase C, PI response and calcium", Otzenhausen, 1–5 September 1985.

Materials and methods

SAC was obtained from Calbiochem-Behring (Cambridge, U.K.), ionomycin from Calbiochem (La Jolla, CA), and TPA, 1,2-diolein, propidium iodide and acridine orange were obtained from Sigma (Poole, Dorset). DiC8 was prepared by the method of Davis *et al.* [8] from dioctanoylphosphatidylcholine which was obtained from Avanti Polar Lipids, Inc. [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]inositol were obtained from Amersham Int. (Amersham, U.K.). The monoclonal antibodies used were as follows: BK19.9 identifies an antigen expressed by rapidly dividing cells which is structurally similar to but serologically distinct from the transferrin receptor. MHM6 identifies the B-lineage restricted CD23p45 antigen also known as Blast-2. A2 recognises the transferrin receptor (Tf-R) and 11EF7 an antigen expressed on activated B lymphocytes. Analysis of surface antigen expression was