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Inhibitory action of brovincamine on catecholamine secretion from cultured bovine adrenal medullary cells: possible relation to its blocking action on Ca^{2+} channels

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Brovincamine has been developed as a new derivative of vincamine, which has been reported to reduce vascular resistance, leading to an increase in the cerebrovascular blood flow [1–3]. Brovincamine is therefore expected to be clinically effective in the treatment of cerebral and cardiac ischemic disorders. Although the pharmacological actions of brovincamine as well as vincamine have been studied, details of the mechanisms underlying these actions remain to be elucidated. Brovincamine has recently been shown to cause a dose-dependent relaxation of the K^+-induced contraction of pulmonary arterial segments, and furthermore found to decrease the duration of slow action potentials in the guinea-pig papillary muscle [4]. Brovincamine therefore seems to produce its vasodilating action through blockade of slow Ca^{2+} channels in cerebral and cardiac blood vessels, but it is still questionable whether the Ca^{2+} antagonistic action of this drug is observable in other types of cell and tissue.

It is well established that Ca^{2+} plays a critical role in the secretory mechanisms of various neurotransmitters and hormones and that catecholamine secretion is initiated by an elevation of the intracellular free Ca^{2+} concentration [5], resulting from the increase in extracellular free Ca^{2+} influx into the cells [6–8]. Catecholamine secretion is therefore a suitable index to reflect an alteration in Ca^{2+} transport across the plasma membrane. Thus, the primary culture of adrenal chromaffin cells is considered to be a useful system to test the effects of various compounds on the Ca^{2+} transport mechanism. To examine whether brovincamine has a blocking action on the Ca^{2+} channels the effects of this drug on both catecholamine secretion and Ca^{2+} influx were investigated in cultured bovine adrenal chromaffin cells.

Materials and Methods

Chromaffin cells were enzymatically prepared from fresh bovine adrenal medulla, plated on 24-well cluster plates at a density of 5×10^5 cells/well, and maintained for 3 or 4 days as previously reported [9].

Cells were washed with 1 mL of balanced salt solution [135 mM NaCl, 5.6 mM KCl, 1.2 mM $MgSO_4$, 2.2 mM $CaCl_2$, 10 mM glucose and 20 mM 4-(2-hydroxyethyl)-1-

piperazineethanesulfonic acid (HEPES), adjusted to pH 7.4], and incubated at 37° for 10 min in 250 μL of balanced salt solution containing different secretagogues with or without the drugs. Catecholamine secretion was determined by the method reported previously [10], and expressed as a percentage of the total cellular catecholamine content secreted into the medium during the incubation period.

In the determination of Ca^{2+} influx, the cells were washed and stimulated by different secretagogues at 37° for 10 min in 250 μL of balanced salt solution containing $^{45}CaCl_2$ (3.0 μCi/mL) with or without the drug. At the end of the incubation period, the medium was removed and the cells were washed three times with 1 mL of ice-cold Ca^{2+}-free balanced salt solution. The cells were solubilized by adding 500 μL of 1% Triton X-100. Radioactivity in the lysate was counted by a liquid scintillation spectrometer. The Ca^{2+} uptake was calculated on the basis of the specific activity of $^{45}Ca^{2+}$ in the incubation mixture.

Brovincamine was a gift from Sandoz Pharmaceuticals (East Hanover, NJ, U.S.A.) and $^{45}CaCl_2$ was purchased from New England Nuclear (Boston, MA, U.S.A.). Verapamil, diltiazem, nifedipine, carbamylcholine and A23187 were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Other chemicals were of commercially available reagent grade.

Results and Discussion

The effect of brovincamine on catecholamine secretion induced by various secretagogues was examined in cultured bovine adrenal chromaffin cells. As shown in Fig. 1, brovincamine inhibited the secretion induced by either carbamylcholine or high K^+, and this inhibitory action was observed to be dependent on its concentration. An approximate 50% inhibition of the secretion induced by carbamylcholine or high K^+ was obtained by 3×10^{-5} M or 1×10^{-4} M drug, respectively. In contrast, the drug failed to cause any significant effect on the secretion induced by a Ca^{2+} ionophore, A23187, at the same concentrations. Brovincamine was found to inhibit the secretion induced by veratridine in a similar manner to that induced by high K^+, while it caused no significant alteration in the basal secretion at any of the concentrations tested here (data not shown). In view of the fact that A23187 can directly stimulate the intracellular secretory system as a result of introducing Ca^{2+} into the cells, these results seem to indicate that the inhibitory action of brovincamine on catecholamine secretion observed here is probably attributable to its blocking action on Ca^{2+} transport across the plasma membrane rather than its disturbing action on the secretory mechanism within the cells. To confirm this possibility, the effect of brovincamine on Ca^{2+} influx into the cells was examined by determining the accumulation of radioactive Ca^{2+} within the cells. It was found that $^{45}Ca^{2+}$ uptake stimulated by either carbamylcholine or high K^+ was significantly inhibited by this drug, under the conditions in which its inhibitory action on the secretory response was observed (Fig. 2). It therefore seems likely that brovincamine inhibits catecholamine secretion through blocking the Ca^{2+} channels in the plasma membrane.

Recently, the inhibitory action of organic and inorganic Ca^{2+} entry blockers on catecholamine secretion has been studied using cat adrenal glands and the Ca^+ channels involved in the secretion have been shown to have differing sensitivities to these blockers [11]. The inhibitory action of brovincamine on the secretion induced by carbamylcholine or high K^+ was determined in the presence of high concentrations of Ca^{2+} using cultured bovine adrenal chromaffin cells and compared with those typical Ca^{2+} entry blockers. As shown in Fig. 3, the high K^+-induced secretion was enhanced by increasing the external Ca^{2+} concentration from 2 to 8 mM. Under these conditions, the high K^+-induced secretion was significantly inhibited by brovincamine and the organic Ca^{2+} entry blockers verapamil and nifedipine. The secretion inhibited by these drugs was shown to be enhanced by increasing the concentration of Ca^{2+} in the incubation mixture. Similarly, catecholamine secretion induced by carbamylcholine was enhanced by elevating the external Ca^{2+} concentration and

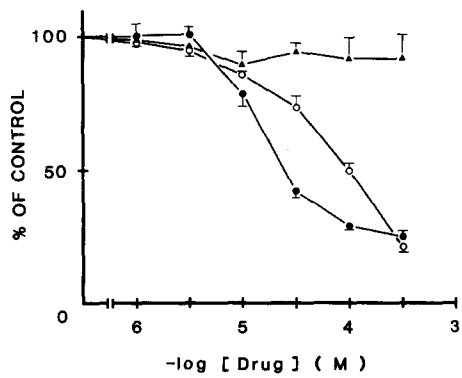

Fig. 1. Effect of brovincamine on catecholamine secretion induced by various secretagogues in primary cultures of adrenal medullary cells. Cells were stimulated by 100 μM carbamylcholine (●), 56 mM KCl (○) and 10 μM A23187 (▲) in the presence of different concentrations of brovincamine, and catecholamine secretion was then determined as described in the text. Results were expressed as per cent of the control (6.95 ± 0.37, 12.03 ± 0.44, and 3.56 ± 0.49% for the carbamylcholine-, high K^+- and A23187-induced secretion, respectively). Values are the mean ± SD (N = 3).

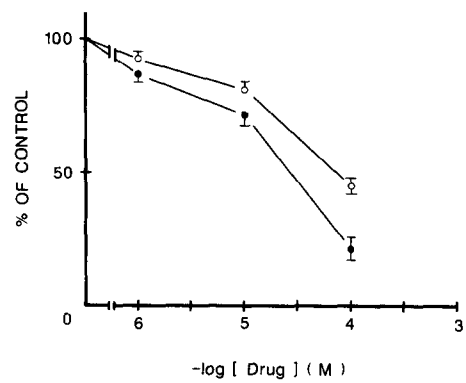

Fig. 2. Effect of brovincamine on Ca^{2+} influx stimulated by various secretagogues in primary cultures of adrenal medullary cells. Cells were stimulated by 100 μM carbamylcholine (●) or 56 mM KCl (○) in a mixture containing $^{45}CaCl_2$ with different concentrations of brovincamine. The uptake was determined as described in the text. Results were expressed as per cent of the control (1.27 ± 0.07 and 3.10 ± 0.11 nmol/10^6 cells for the carbamylcholine- and high K^+-stimulated uptake respectively). Values are the mean ± SD (N = 3).

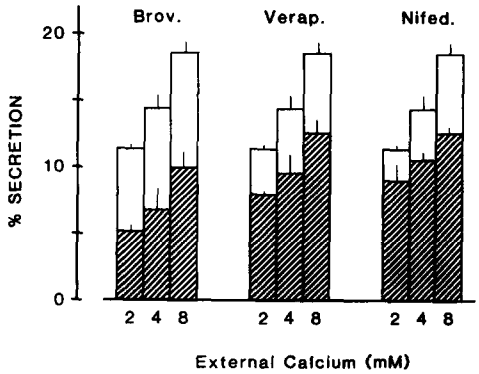

Fig. 3. Inhibitory actions of brovincamine and various Ca^{2+} entry blockers on catecholamine secretion induced by high K^+ in primary cultures of adrenal medullary cells. Cells were incubated in the high K^+-mixture containing different concentrations of Ca^{2+} with (hatched column) or without (open column) 100 μM brovincamine, 10 μM verapamil or 10 μM nifedipine, and the secretion was then determined as described in the text. Results were calculated by subtracting the basal level from the secretion stimulated by high K^+. Values are the mean ± SD (N = 3).

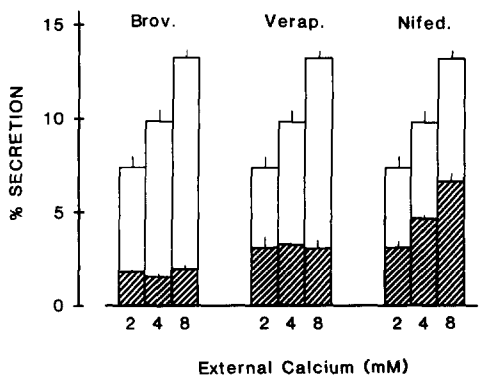

Fig. 4. Inhibitory actions of brovincamine and various Ca^{2+} entry blockers on catecholamine secretion induced by carbamylcholine in primary cultures of adrenal medullary cells. Cells were incubated with 100 μM carbamylcholine in an incubation mixture containing different concentrations of Ca^{2+} with (hatched column) or without (open column) 100 μM brovincamine, 10 μM verapamil or 10 μM nifedipine. Catecholamine secretion was determined as described in the text. Results were obtained by subtracting the basal secretion from the secretion stimulated by carbamylcholine. Values are the mean ± SD (N = 3).

was inhibited by brovincamine or organic Ca^{2+} entry blockers. In contrast to the high K^+-induced secretion, the secretion inhibited by either brovincamine or verapamil was not enhanced by increasing the external Ca^{2+} concentration, whereas the secretion inhibited by nifedipine was enhanced in this way (Fig. 4). Similarly to nifedipine, the secretion inhibited by diltiazem was enhanced by increasing the Ca^{2+} concentration (data not included in Fig. 4). It therefore seems reasonable to conclude that the inhibitory action of brovincamine on catecholamine secretion is probably attributable to its blocking action on the Ca^{2+} channels, which may be similar to the inhibition caused by verapamil.

In summary, the present study showed that brovincamine inhibited catecholamine secretion as a consequence of blocking the Ca^{2+} influx into cultured bovine adrenal chromaffin cells, and this inhibition was similar to that caused by a Ca^{2+} entry blocker, verapamil. It has been previously reported that brovincamine has a vasodilating action on isolated cardiovascular preparations due to its blocking action on the slow Ca^{2+} channels in blood vessels [4]. In view of these findings, it seems reasonable to conclude that brovincamine may cause the vasodilation *in situ*, presumably through the inhibition of neurotransmitter release from the adrenergic nerve. Thus, the inhibition of Ca^{2+} channels in the adrenergic nerve ending, as well as the blood vessel, is considered to be a possible mechanism for the therapeutic action of brovincamine on cerebral and cardiac ischemic disorders.

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