REFERENCES

1. P. Tyrer, *Br. J. Psychiat.* **128**, 354 (1976).
2. J. M. Davis, *Archs gen. Psychiat.* **13**, 552 (1965).
3. J. M. Davis, *Am. J. Psychiat.* **133**, 1 (1976).
4. E. D. Hendley and S. H. Snyder, *Nature, Lond.* **220**, 1330 (1968).
5. H. C. Kim, and A. D'Iorio, *Can. J. Biochem.* **46**, 295 (1968).
6. M. B. H. Youdim, G. G. S. Collins and M. Sandler, *Nature, Lond.* **223**, 626 (1969).
7. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
8. J. Knoll and K. Magyar, *Adv. biochem. Psychopharmac.* **5**, 393 (1972).
9. S. Lipper, D. L. Murphy, S. Slater and M. S. Buchsbaum, *Psychopharmacology* **62**, 123 (1979).
10. J. Mann and S. Gershon, *Life Sci.* **26**, 877 (1980).
11. J. A. Herd, *Clin. Trials J.* **6**, 219 (1969).
12. J. S. Carman, J. C. Gillin, D. L. Murphy, D. R. Weinberger, J. E. Kleinman, L. B. Biegelo and R. J. Wyatt, *Commun. Psychopharmac.* **2**, 513 (1978).
13. J. Mendlewicz and M. B. H. Youdim, *J. Neural Transm.* **43**, 279 (1978).
14. A. J. Azzaro and D. J. Smith, *J. Neurochem.* **24**, 811 (1975).
15. V. P. Whittaker, J. A. Michaelson and R. J. Kirkland, *Biochem. J.* **90**, 293 (1964).
16. J. T. Coyle and S. H. Snyder, *J. Pharmac. exp. Ther.* **170**, 221 (1969).
17. E. Layne, *Meth. Enzym.* **3**, 447 (1954).
18. A. J. Azzaro and D. J. Smith, *Neuropharmacology* **16**, 349 (1977).
19. J. T. Litchfield and F. Wilcoxon, *J. Pharmac. exp. Ther.* **96**, 99 (1949).
20. K. T. Demarest, D. J. Smith and A. J. Azzaro, *J. Pharmac. exp. Ther.* **215**, 416 (1980).
21. J. C. K. Lai, T. K. C. Leung, J. F. Guest, L. Lin and A. N. Davison, *Biochem. Pharmac.* **29**, 2763 (1980).
22. J. Knoll, *J. Neural Transm.* **43**, 177 (1978).

Biochemical Pharmacology, Vol. 31, No. 12, pp. 2197–2199, 1982.
Printed in Great Britain.

0006-2952/82/122197–03 $03.00/0

Potassium release by α_2-adrenergic receptor stimulation of rat parotid acinar cells

(*Received* 8 *June* 1981; *accepted* 9 *December* 1981)

A classification of α-adrenergic receptors [1–3] has been developed based on the abilities of certain selective agents to preferentially elicit or block α-adrenergic responses. The agonists clonidine and α-methyl norepinephrine and the antagonist yohimbine are more potent at α_2-adrenergic receptors, while the agonist phenylephrine and the antagonists WB-4101 and prazosin are more potent at α_1 receptors. Fain and Garcia-Sainz [4] noted that known α_1-adrenergic receptors appear to mediate responses by facilitating the entry of extracellular calcium into target cells, while α_2-adrenergic receptors probably mediate responses by inhibiting cAMP* formation.

Parotid acinar cells release K^+ when exposed to α-adrenergic agonists. K^+ release appears to result from Ca^{2+} entry since the response is Ca^{2+} dependent and can be elicited by Ca^{2+} ionophores [5]. Radioligand binding studies of rat submaxillary glands have shown the presence of receptor binding sites with both α_1 [6] and α_2[6, 7] characteristics, while the binding sites in parotid have not been classified. Accordingly, we undertook a characterization of the α-adrenergic response in dispersed parotid cells with selective antagonists in order to test the hypothesis that Ca^{2+} entry is mediated by α_1-adrenergic receptors.

Materials and methods

Cell preparation. Dispersed parotid cells were prepared as described previously [8, 9]. Freshly excised parotid glands were minced and subsequently incubated in collagenase and hyaluronidase for 1 hr with gentle mechanical disruption. After filtration through nylon mesh, the cells were washed twice and resuspended in Hanks–Hepes* buffer [8] (143.3 mM NaCl, 5.4 mM KCl, 0.81 mM $MgSO_4 \cdot 7H_2O$, 2.4 mM $CaCl_2$, 20.0 mM Hepes, and 5.6 mM dextrose) gassed with 95% O_2–5% CO_2. Approximately 1–2 $\times 10^8$ cells were routinely obtained from the parotid glands of five or six male Sprague–Dawley rats (Zivic—Miller Laboratories, Allison Park, PA).

Potassium release. K^+ release was measured in 50 μl of cells (approximately 5×10^6 cells) suspended in gassed Hanks–Hepes buffer at 37°. In most experiments, epinephrine alone or in combination with an antagonist in Hanks–Hepes buffer was added directly to the cell preparation. After 30 sec of incubation, the suspension was centrifuged (Beckman microfuge) for an additional 30 sec and 25 μl of the supernatant fraction was diluted with 1 ml of an Acationox (Scientific Products, Charlotte, NC) solution. K^+ was measured with an atomic absorption spectrometer (Perkin–Elmer 303). In some experiments, the cells were preincubated for 30 min in buffer containing one of the α-adrenergic antagonists before being exposed to epinephrine for 30 sec.

High concentrations of prazosin were insoluble in buffer alone. A stock solution of prazosin (10 μM) was routinely prepared in buffer with 10% ethanol. This stock solution was diluted with buffer alone. Control experiments with 10% ethanol showed that neither this concentration nor more dilute solutions of ethanol had any effect on the K^+ release elicited by epinephrine from intact cells.

Materials. WB-4101 was donated by WB Pharmaceuticals, Ltd., Berkshire, U.K.; prazosin HCl was supplied by Pfizer, Inc., Brooklyn, NY, U.S.A.; and yohimbine HCl

* Abbreviations: cAMP, cyclic 3′,5′-adenosine monophosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethyleneglycolbis(aminoethylether)tetra-acetate; and WB-4101, (2-[2-(2′,6′-dimethoxyphenoxy)ethylaminomethyl]-1,4-benzodioxane HCl.

was purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. Clonidine was a gift of Boehringer, Ingelheim am Rhein, West Germany, and phentolamine was a gift of Ciba–Geigy, Inc., Summit, NJ, U.S.A. All other materials were of the greatest purity commercially obtainable.

Results

As in previous studies [8, 9], exposure to (−)epinephrine elicited a rapid and transient release of K^+ from parotid acinar cells with an EC_{50} of 4 μM (Fig. 1). As expected, incubation with yohimbine, WB 4101 or prazosin alone had no effect on acinar cells (data not shown). To determine the potency of these agents, 100 μM (−)epinephrine was chosen to stimulate the cells since that concentration gave a maximal and reproducible K^+ release. A 1 mM concentration of (−)epinephrine consistently produced the same or slightly less K^+ release [8] (data not shown). All three agents inhibited epinephrine (Fig. 2). The relative potencies were yohimbine > WB 4101 > prazosin. In fact, yohimbine was the only antagonist capable of inhibiting greater than 50% of the K^+ release elicited by 100 μM (−)epinephrine. The same potency series pertained when a submaximal concentration of 10 μM (−)epinephrine was studied. The abilities of yohimbine, prazosin and WB 4101 to block epinephrine-induced K^+ release were not altered by preincubation of the cells with the antagonists. For example, even after a 30-min preincubation, WB 4101 was unable to inhibit greater than 50% of the K^+ release from a 30-sec exposure to (−)epinephrine.

The possibility that yohimbine might block K^+ release by some mechanism other than receptor antagonism was raised by its marked potency in blocking epinephrine. Since the conditions for K^+ release differed slightly from our previous studies [9], the well-established Ca^{2+} dependency of K^+ release was confirmed under the conditions used in the yohimbine experiments. EGTA (10 mM) was included with the 100 μM epinephrine and added to parotid acinar cells for 30 sec. No K^+ release was observed. The same suspension of cells exposed to epinephrine without EGTA released 1.8 ± 0.3 μg K^+/25 μl cells (55% of the total cellular K^+).

Since carbachol appears to elicit K^+ release using the same Ca^{2+} entry mechanism as epinephrine [5, 8], the possibility that yohimbine had a non-specific effect on K^+ release was tested by exposing the cells to carbachol. These cells released K^+ when exposed to 100 μM carbachol for

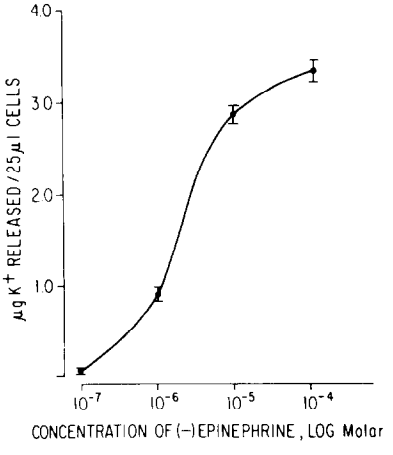

Fig. 1. α-Adrenergic response in parotid acinar cells. The amount of K^+ released from a suspension of parotid acinar cells exposed to (−)epinephrine is shown. Each point is the mean of five experiments carried out in triplicate. The vertical bars represent one S.E.M.

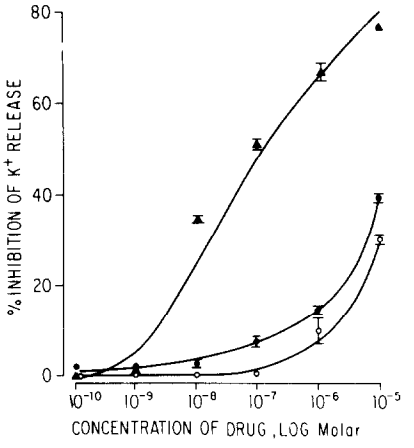

Fig. 2. Inhibition of the α-adrenergic response by selective α-adrenergic antagonists. The percent inhibitions of K^+ release from cells exposed to 100 μM (−)epinephrine by various concentrations of yohimbine (▲), WB 4101 (●) and prazosin (○) are shown. Each point is the mean of two or three experiments carried out in triplicate.

30 sec (3.2 ± 0.2 μg K^+/25 μl cells; 92% of total K^+). Preincubation with 1 μM yohimbine did not affect the ability of carbachol to elicit K^+ release (3.3 μg K^+/25 μl), while 100 μM yohimbine reduced the release slightly (2.7 μg K^+/25 μl). By contrast 1 μM yohimbine inhibited almost 70% of the K^+ released by 100 μM epinephrine (Fig. 2).

Discussion

The central finding in these experiments is that in parotid acinar cells yohimbine is a more potent antagonist of epinephrine-induced K^+ release than WB 4101 or prazosin. By present classification schemes these data suggest that the α-adrenergic response in these cells is mediated by an α_2-adrenergic receptor. Since the K^+ release in parotid depends on the presence of external Ca^{2+}, these experiments suggest that an α_2-adrenergic receptor can facilitate Ca^{2+} entry. The parotid α_2-adrenergic response appears to differ from that of several other tissues where α_2-adrenergic stimulation leads to inhibition of cAMP formation [4]. An α-adrenergic inhibition of cAMP formation in parotid slices has been noted, but it appears to require Ca^{2+} [10].

There is no evidence to link inhibition of cAMP formation with K^+ release in these cells. The time course of K^+ release studied in these experiments was rapid, being complete by 30 sec [9], while cAMP accumulation appears to be a slower phenomenon requiring 5 min to be maximum [10, E. Stanton and J. N. Davis, unpublished observations]. α-Adrenergic inhibition of cAMP formation has been demonstrated in parotid slices, but not in dispersed cells. The slice could contain other cellular elements besides acinar cells. Despite the lack of evidence, we cannot exclude the possibility that the α_2-adrenergic inhibition of cAMP formation is related to K^+ release in some way.

It should be noted that we have studied K^+ released by epinephrine in 30 sec. Studies of parotid slices have suggested that K^+ release may be complicated at later time points when changes in membrane K^+ pumping and an uptake of Ca^{2+} into vesicles take place [5]. There could be α_1-adrenergic receptors on these cells which elicit K^+ release at a slower rate than the rapid response we studied. α_1-Adrenergic receptors clearly facilitate Ca^{2+} entry in some tissues [4]. These experiments demonstrate that, under certain conditions in the parotid, α_2-adrenergic receptors elicit K^+ release, a response dependent on the presence of external Ca^{2+}. This result suggests that α_2-adrenergic receptors might facilitate Ca^{2+} entry.

In summary, α-adrenergic stimulation elicited a rapid release of K^+ from dispersed parotid acinar cells in the presence of external Ca^{2+}. A recent classification of α-adrenergic receptors proposed that α_1 receptors facilitate Ca^{2+} entry while α_2 receptors interact with adenylate cyclase. We tested this proposal by studying the ability of selective α-adrenergic antagonists to prevent epinephrine-induced release of K^+ from parotid cells. The α_1 agents, WB 4101 and prazosin, were less potent than the α_2 antagonist yohimbine. These data suggest that α_2-adrenergic receptors in the parotid might facilitate the entry of Ca^{2+}.

Neurology Research Laboratory
Veterans Administration Medical Center
Durham, NC 27705, and
Departments of Medicine (Neurology) and Pharmacology
Duke University Medical Center
Durham, NC 27710, U.S.A.

GERALD D. MILLER
JAMES N. DAVIS*

* Send all correspondence to: Dr. James N. Davis, Neurology Section, VA Medical Center, Durham, NC 27705.

REFERENCES

1. S. Z. Langer, *Biochem. Pharmac.* **23**, 1793 (1974).
2. S. Berthelsen and W. A. Pettinger, *Life Sci.* **21**, 595 (1977).
3. C. L. Wood, C. D. Arnett, W. R. Clarke, B. S. Tsai and R. J. Lefkowitz, *Biochem. Pharmac.* **28**, 1277 (1979).
4. J. N. Fain and J. A. Garcia-Sainz, *Life Sci.* **26**, 1183 (1980).
5. J. W. Putney, *Pharmac. Rev.* **30**, 209 (1979).
6. D. C. U'Prichard and S. H. Snyder, *Life Sci.* **24**, 79 (1979).
7. C. D. Arnett and J. N. Davis, *J. Pharmac. exp. Ther.* **211**, 294 (1979).
8. J. N. Davis, E. Olender, W. Maury and R. McDaniel, *Molec. Pharmac.* **18**, 356 (1980).
9. W. J. Strittmater, J. N. Davis and R. J. Lefkowitz, *J. biol. Chem.* **252**, 5472 (1977).
10. Y. Oron, J. Kellogg and J. Larner, *Molec. Pharmac.* **14**, 1018 (1978).

Biochemical Pharmacology, Vol. 31, No. 12, pp. 2199–2200, 1982.
Printed in Great Britain.

0006-2952/82/122199-02 $03.00/0

Effect of haloperidol pre- and post-treatment on the ability of pergolide and bromocriptine to antagonize the γ-butyrolactone-induced increase in brain dopamine in rats

(*Received* 10 *August* 1981; *accepted* 11 *January* 1982)

Recently, Marek and Roth [1] reported that the ability of dopamine agonists to counteract the increase in dopamine formation produced by administration of γ-butyrolactone (GBL) was prevented by haloperidol under some but not all conditions. GBL blocks impulse flow and causes accumulation of dopamine, which is counteracted by dopamine agonists acting presynaptically. Haloperidol given prior to the dopamine agonists prevented the effects of apomorphine and bromocriptine, whereas haloperidol given after the dopamine agonist prevented the effect of apomorphine but not of bromocriptine. Marek and Roth [1] suggested that bromocriptine may interact irreversibly or noncompetitively with presynaptic dopamine receptors. The experiments described here compare the effects of pergolide, a new potent dopamine agonist [2–5] being used in the treatment of Parkinson's disease [6] and hyperprolactinemia [7]. Pergolide is an ergoline that shows potent dopamine agonist activity *in vitro* [3–5] and *in vivo* [2–4] and has a long duration of action [2–4]. Since Bannon *et al.* [8] have suggested that irreversible interactions with dopamine receptors may account for the long duration of some ergots, we were interested in evaluating whether pergolide resembled bromocriptine in respect to reversibility by post-treatment with haloperidol.

The experimental design was based on the work of Marek and Roth [1], but we measured dopamine rather than the accumulation of dopa following decarboxylase inhibition to avoid having to give a fourth drug, the decarboxylase inhibitor. Our measurements were made in whole brain whereas Marek and Roth [1] had measured in two brain regions, the striatum and the olfactory tubercle. In our studies, male Wistar rats weighing 140–210 g were obtained from Harlan Industries, Cumberland, IN. GBL (Eastman Chemical Products, Kingsport, TN) was injected at a dose of 500 mg/kg i.p., 35 min before the rats were killed. Pergolide mesylate (Lilly Research Laboratories, Indianapolis, IN), 0.3 mg/kg i.p., and bromocriptine mesylate (Sandoz Pharmaceuticals, East Hanover, NJ), 10 mg/kg i.p., were injected 1 hr prior to GBL. Haloperidol (McNeil Laboratories, Fort Washington, PA), 1 mg/kg i.p., was injected 5 min before or 50 min after pergolide or bromocriptine. Rats were decapitated, and whole brains were quickly removed, frozen on dry ice, and stored at −15° prior to analysis. Dopamine concentration in whole brain was measured by liquid chromatography with electrochemical detection [9].

The results are shown in Fig. 1. Dopamine concentration was increased ($P < 0.1$) by GBL injection (compare two left columns). The injection of either pergolide or bromocriptine (third and sixth columns from left) significantly antagonized the accumulation of dopamine. Haloperidol given before bromocriptine completely prevented the antagonistic effect of bromocriptine (second column from right), but haloperidol given subsequent to