

## Editorial

## Calcium-channel blockers and asthma

The concentration of free calcium ions ( $\text{Ca}^{++}$ ) in the cytoplasm regulates many aspects of cell function, including muscle contraction and the secretion of mediators, neurotransmitters and hormones. Contraction of airway smooth muscle, secretion of mast cell mediators and mucus, and vagal neurotransmission, all of which may be increased in asthma, are dependent on movement of  $\text{Ca}^{++}$  into the cell. This raises the possibility that an increase in  $\text{Ca}^{++}$  flux may be a fundamental abnormality in asthma and might be an explanation for bronchial hyperreactivity.<sup>1</sup> The development of drugs that interfere with the entry of  $\text{Ca}^{++}$  into cells has now made it possible to study the role of  $\text{Ca}^{++}$  in disease, and these drugs are widely used in the treatment of cardiovascular disease. There has recently been increasing interest in the use of calcium-channel blockers in asthma, not because of their therapeutic potential but because of what they may reveal about underlying mechanisms.

## CALCIUM IONS AND CELL FUNCTION

The concentration of free intracellular  $\text{Ca}^{++}$  in resting cells is approximately  $0.1 \mu\text{mol/l}$ , whereas that in extracellular fluid is  $1 \text{ mmol/l}$ ;<sup>2,3</sup> and recent studies using bioluminescent indicators of free intracellular  $\text{Ca}^{++}$  have found that the  $\text{Ca}^{++}$  concentration increases 100-fold after cell activation.<sup>4,5</sup> When intracellular  $\text{Ca}^{++}$  rises to a critical concentration (about  $1 \mu\text{mol/l}$ ) it binds to a specific binding protein called calmodulin, which then activates the specific kinase and enzyme system that brings about the response, such as contraction or secretion. This increase in cytoplasmic  $\text{Ca}^{++}$  is derived from extracellular  $\text{Ca}^{++}$  or intracellular stores (mainly sarcoplasmic reticulum), or both. Indeed, the entry of  $\text{Ca}^{++}$  into the cell may trigger the release of  $\text{Ca}^{++}$  from intracellular stores. The source of  $\text{Ca}^{++}$  required for the cellular response may differ from tissue to tissue. The cell membrane itself is impermeable to  $\text{Ca}^{++}$  and  $\text{Ca}^{++}$  moves into the cell through specific channels. These are macromolecular structures traversing the membrane

Address for reprint requests: Dr P J Barnes, Department of Medicine (Respiratory Division), Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0HS.

which selectively permit  $\text{Ca}^{++}$  to enter the cell down its concentration gradient. There are at least two types of calcium channel, one opening in response to cellular depolarisation (voltage-dependent) and one in response to activation of a specific receptor, such as an  $\alpha$ -adrenoceptor (receptor-operated).<sup>6</sup> It has been calculated that there are up to 10 000 voltage-dependent calcium channels in each cardiac cell.<sup>3</sup>

The interrelationships between  $\text{Ca}^{++}$  and cyclic nucleotides are complex. In smooth muscle cyclic AMP accelerates  $\text{Ca}^{++}$  removal from the cytoplasm and prevents the influx of  $\text{Ca}^{++}$ , thus reversing the effects of activation.<sup>7</sup>  $\text{Ca}^{++}$  also inhibits adenylate cyclase and stimulates guanylate cyclase.<sup>8</sup> Many of the drugs used in treating asthma may reduce intracellular  $\text{Ca}^{++}$ . Thus  $\beta$ -adrenoceptor agonists bring about relaxation by increasing cyclic AMP and cholinergic antagonists by directly reducing  $\text{Ca}^{++}$  influx by reducing  $\text{Ca}^{++}$  in the smooth muscle cell. Theophyllines and cromoglycate may also have their primary actions on  $\text{Ca}^{++}$  influx.

## CALCIUM-CHANNEL BLOCKERS

Voltage-dependent calcium channels are specific to calcium, although cations of similar size such as barium may also enter. Larger cations, such as manganese and lanthanum, block the channel. Recently organic compounds that specifically block these channels have been developed; they include verapamil, nifedipine, and diltiazem, which have no apparent structural similarities.<sup>9</sup> Binding studies using labelled derivatives indicate that these drugs bind with high affinity to different sites on the calcium channel<sup>9a,10</sup> but there may be complex allosteric interactions between the different classes of calcium-channel blocker.<sup>10a</sup> Their precise mode of action at a molecular level is still debated.

Calcium-channel blockers are now extensively used in cardiac disease, and appear to be safe and without troublesome side effects.<sup>9,11</sup> These drugs appear to be relatively specific for voltage-dependent calcium channels, and have little action on receptor-operated channels. At higher doses they may have other effects, such as  $\alpha$ -adrenoceptor

blockade, phosphodiesterase inhibition, and inhibition of Na-K ATPase, and may not be as selective as previously believed.<sup>12</sup>

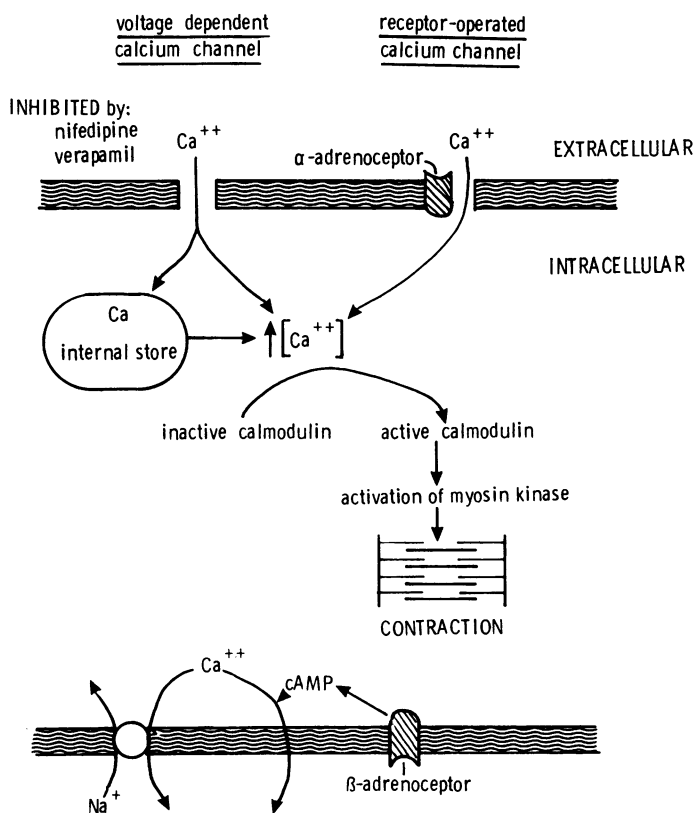
#### AIRWAY SMOOTH MUSCLE

The source of calcium for muscle contraction differs between muscle types, and even between the same muscle type in different tissues. Skeletal muscle is dependent only on intracellular calcium stores and contraction is therefore unaffected by calcium-channel blockers, whereas vascular smooth muscle is dependent on extracellular  $Ca^{++}$  and is therefore susceptible to blockade. The dependency of smooth muscle on extracellular  $Ca^{++}$  may vary between different sites since smooth muscle in arterioles is much more sensitive to calcium-channel blockers than is that from veins.<sup>12</sup>

Airway smooth muscle has been less extensively studied and appears to be dependent on both external and internal sources of  $Ca^{++}$ , but this may vary with the contractile stimulus (fig). Serotonin-induced contraction of canine tracheal strips is strongly inhibited by lanthanum and by verapamil, whereas acetylcholine-induced contraction is rela-

tively resistant to blockade.<sup>13</sup> In the same preparation it was found that verapamil inhibited contraction due to low concentrations of acetylcholine but not contraction due to high concentrations.<sup>14</sup> Similar results have been reported with guinea-pig trachea,<sup>15</sup> suggesting that the relative contribution of intracellular and extracellular  $Ca^{++}$  may depend not only on the stimulus but also on its magnitude. In vitro nifedipine inhibits contraction induced by histamine and by antigen in guinea-pig trachea<sup>16-17</sup> and human airway smooth muscle.<sup>18-19</sup> Since nifedipine is equally potent in guinea-pig lung strip preparations, this suggests that small airways may behave in the same way.<sup>16</sup> Animal studies in vivo have shown that nifedipine given intravenously or by inhalation has no effect on resting bronchomotor tone,<sup>16-20-21</sup> but gives appreciable protection against bronchoconstriction induced by histamine, prostaglandin  $F_{2\alpha}$ , and citric acid in dogs,<sup>20-21</sup> and against constriction induced by histamine and carbachol in guinea-pigs.<sup>16</sup>

Similar findings have been reported in patients with asthma. Neither nifedipine given sublingually nor verapamil given by inhalation had any significant effect on resting airway function.<sup>22-25</sup>



*Calcium ion ( $Ca^{++}$ ) fluxes in an airway smooth muscle cell. The rise in intracellular  $Ca^{++}$  causes contraction and may be due to entry of  $Ca^{++}$  from outside via voltage-dependent or receptor-operated calcium channels or from internal stores. Only the voltage-dependent calcium channel is effectively inhibited by calcium-channel blockers, such as nifedipine and verapamil.  $Ca^{++}$  is removed from the cell by an  $Na^+ - Ca^{++}$  exchange pump or by a cyclic-AMP-dependent mechanism.*

There was, however, significant protection against bronchoconstriction induced by exercise,<sup>22-24</sup> hyperventilation,<sup>26</sup> histamine,<sup>23 25 27</sup> methacholine,<sup>27</sup> deep inspiration,<sup>28</sup> and antigen,<sup>18</sup> although in one study no protection against histamine-induced bronchoconstriction was found.<sup>29</sup> In only one study was protection completely effective,<sup>22</sup> the others showing partial protection with considerable inter-subject variability. Perhaps the dose of calcium-channel blocker used in these studies was too low, but the dose of sublingual nifedipine is limited by cardiovascular side effects and possibly administration by aerosol (as in some of the animal studies) would be more effective. It is also possible that induced bronchoconstriction in asthma is regulated by receptor-operated rather than voltage-dependent calcium channels, and it may be more dependent on internal calcium stores. Further studies on the calcium dependency of human airway smooth muscle are required before these questions can be answered.

An interesting aspect of these findings is that calcium-channel blockers appear to change the bronchoconstrictor response without causing bronchodilation. The long-term effects of calcium-channel blockers on bronchial reactivity are not yet known, although a small improvement in airway function has been reported in some patients with labile airways obstruction after two weeks of treatment.<sup>30</sup>

#### MAST CELLS

It has long been recognised that extracellular  $Ca^{++}$  is necessary for antigen-induced mediator release.<sup>31</sup> The calcium ionophore A-23187 (which carries calcium into the cell) and the direct intracellular injection of calcium stimulate histamine release from rat peritoneal mast cells, the amount released being proportional to the calcium influx.<sup>32</sup> It is proposed that bridging of IgE receptors increases phospholipid methylation in the cell membrane, which leads to  $Ca^{++}$  influx.<sup>33</sup> Lanthanum and methoxy-verapamil inhibit IgE-mediated histamine release from rat peritoneal cells<sup>34 35</sup> and nifedipine passive cutaneous anaphylaxis in rat skin.<sup>36</sup>

Mediator secretion from human lung is also dependent on extracellular  $Ca^{++}$ <sup>37</sup> and recently both verapamil and nifedipine have been found to inhibit IgE-mediated secretion of slow-reacting substance of anaphylaxis (SRS-A) from passively sensitised human lung fragments,<sup>38 39</sup> although the concentrations of drug used were high and there was little or no effect on histamine secretion. Nifedipine has also been shown to inhibit secretion of SRS-A and platelet-activating factor by human polymorphs,<sup>40</sup> and the release of histamine from human basophils

(SP Skov *et al*, paper presented to Eleventh International Congress of Allergology and Clinical Immunology, 1982). In vivo, nifedipine reduces plasma histamine concentrations and prevents the rise in plasma histamine with exercise in individuals with asthma,<sup>23</sup> although this is likely to reflect mediator release from circulating basophils rather than from pulmonary mast cells.

#### OTHER PULMONARY EFFECTS

Neurotransmission is similarly dependent on external  $Ca^{++}$  and it is possible that  $Ca^{++}$  channel blockers have an inhibitory effect on reflex vagal bronchoconstriction, although this has not been studied systematically. The secretory activity of airway submucosal glands and transport of ion and water across the airway epithelium are also dependent on external  $Ca^{++}$ ,<sup>41</sup> but the effects of calcium-channel blockers on airway gland secretion are not known. Nifedipine has an inhibitory action on acute hypoxic pulmonary vasoconstriction, which may be beneficial in pulmonary hypertension secondary to chronic lung disease;<sup>42</sup> but this effect is potentially disadvantageous in asthma since it might increase ventilation-perfusion mismatching, particularly during an acute exacerbation. In anaesthetised dogs calcium-channel blockers have been shown to have a more potent effect on tracheal blood flow than on airway smooth muscle,<sup>43</sup> raising the possibility that these agents cause an increase in airway blood flow in asthma, which might lead to increased vascular leakage and mucosal oedema. Chemotaxis of inflammatory cells is also a calcium-dependent process<sup>44</sup> and it is possible that calcium-channel blockers might have an effect on the inflammatory response in asthma. These possibilities have not yet been explored.

#### FUTURE DEVELOPMENTS

Experimental studies have shown that airway smooth muscle and pulmonary mast cells are dependent on external sources of  $Ca^{++}$  and that calcium-channel blockers protect against induced bronchoconstriction and possibly mediator release. But these effects are disappointingly small and the reasons for this require further elucidation. The limitation of the dose that can be given systematically is one possible explanation, although the doses used do have appreciable actions on peripheral blood vessels. The sensitivity of a tissue to calcium-channel blockers will depend on its requirement for extracellular  $Ca^{++}$ . Airway smooth muscle appears to use both internal and external sources, so that it is less sensitive to calcium-channel blockers than vascular smooth muscle.<sup>43</sup> Since the source of  $Ca^{++}$  for airway smooth muscle contraction depends on the

contractile stimulus and even its magnitude, the effects of calcium-channel blockers are likely to be variable. Furthermore, the role of receptor-operated calcium channels, which are not susceptible to conventional calcium-channel blockers, requires further study. There are also differences between species. For example, rat vessels are more sensitive to blockers than those of the cat or rabbit.<sup>12</sup> More work on human airways is needed.

There are also differences in potency and effects between the various calcium-channel blockers available. The demonstration that each class of calcium-channel blocker binds to a different site in the calcium channel raises the possibility that combinations of different blockers may be more effective. Furthermore, it is possible that derivatives of existing blockers, such as nisoldipine, nitrendipine, and nimodipine, or new classes of blocker might be more potent or have a more selective action on airway smooth muscle or mast cells. Other drugs which interfere with  $\text{Ca}^{++}$  availability may also prove to be of value. Drugs similar to methylene dioxyindenes which block intracellular calcium release might eventually prove to be beneficial, although drugs now available are likely to be too toxic for clinical use.<sup>45</sup> Several drugs are known to affect calmodulin binding, although they lack specificity.<sup>46</sup> Studies on calcium-channel blockers and the role of abnormal  $\text{Ca}^{++}$  flux in the pathogenesis of asthma are only just beginning and future developments are likely to be exciting as new drugs become available.

A practical message from the existing studies is that calcium-channel blockers are safe to use in asthma. Since they are effective treatment for angina and hypertension,<sup>8,9,11</sup> they may be the treatment of choice for these conditions in patients who also have asthma and chronic airway obstruction, when  $\beta$ -adrenoceptor antagonists are contraindicated.

## References

- Middleton E. Antiasthmatic drug therapy and calcium ions: review of pathogenesis and role of calcium. *J Pharm Sci* 1980;**69**:243-51.
- Borle AB, Snowdowne KW. Measurement of intracellular free calcium in monkey kidney cells with aequorin. *Science* 1982;**217**:252-4.
- Reuter H. Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature* 1983;**301**:569-74.
- Morgan JP, Morgan KG. Vascular smooth muscle: the first recorded  $\text{Ca}^{++}$  transients. *Pfuegers Arch* 1982;**395**:75-7.
- Tsien RY. A non-disruptive technique for loading calcium buffers and indicators into cells. *Nature* 1981;**290**:527-8.
- Berridge M J. Receptor-operated calcium channels. *Handbook Exp Pharmacol* 1982;**58**:227-70.
- Adelstein RS, Hathaway DR. Role of calcium and cyclic adenosine 3':5' monophosphate in regulating smooth muscle contraction. *Am J Cardiol* 1979;**44**:783-7.
- Triggle DJ, Swamy VC. Pharmacology of agents that affect calcium: agonists and antagonists. *Chest* 1980;**78**, suppl:174-9.
- Braunwald E. Mechanism of action of calcium channel blocking agents. *N Engl J Med* 1982;**307**:1618-27.
- Holck M, Thornes S, Haeusler G. Characterization of [<sup>3</sup>H]nifedipine binding sites in rabbit myocardium. *Eur J Pharmacol* 1982;**85**:305-15.
- Murphy KMM, Gould RJ, Largent BL, Snyder SH. A unitary mechanism of calcium antagonist drug action. *Proc Natl Acad Sci USA* 1983;**80**:860-4.
- Lee KS, Tsien RW. Mechanism of calcium channel blockade by verapamil, D 600, diltiazem, and nitrendipine in single dialysed heart cells. *Nature* 1983;**302**:790-4.
- Dargie H, Rowland E, Krikler D. Role of calcium antagonists in cardiovascular therapy. *Br Heart J* 1981;**46**:8-16.
- Smith RD. Calcium entry blockers: key issues. *Fed Proc* 1983;**42**:201-6.
- Coburn RF. The airway smooth muscle cell. *Fed Proc* 1977;**36**:2692-7.
- Farley JM, Miles PR. The sources of calcium for acetylcholine-induced contractions of dog tracheal smooth muscle. *J Pharmacol Exp Ther* 1978;**207**:340-6.
- Creese BR, Denborough MA. Sources of calcium for contraction of guinea-pig isolated tracheal smooth muscle. *Clin Exp Pharm Physiol* 1981;**8**:175-82.
- Fanta CH, Venugopal CS, Lacoutre PG, Drazen M. Inhibition of bronchoconstriction in the guinea pig by a calcium channel blocker, nifedipine. *Am Rev Respir Dis* 1982;**125**:61-6.
- Weiss EB, Markowicz J. Inhibition of anaphylaxis in airway smooth muscle by calcium channel drugs, verapamil and nifedipine. *Am Rev Respir Dis* 1981;**123**:42 (abstract).
- Henderson AF, Dunlop LS, Costello JF. Effect of nifedipine on antigen-induced bronchoconstriction. *Am Rev Respir Dis* 1983 (in press).
- Drazen JM, Fanta CH, Lacoutre PG. Effect of nifedipine on constriction of human tracheal strips in vitro. *Br J Pharmacol* 1983;**78**:687-91.
- Malo PE, Wasserman MA, Griffin RL. Effect of intravenous and aerosol nifedipine on prostaglandin  $\text{F}_{\alpha\alpha}$  and histamine-induced bronchoconstriction in anesthetized dogs. *J Pharmacol Exp Ther* 1982;**221**:410-5.
- Brugman TM, Darnell ML, Hirschman CA. Nifedipine aerosol attenuates airway constriction in dogs with hyperreactive airways. *Am Rev Respir Dis* 1983;**127**:14-7.
- Cerrina J, Denjean A, Alexandre G, Lockhart A, Duroux P. Inhibition of exercise-induced asthma by a calcium antagonist, nifedipine. *Am Rev Respir Dis* 1981;**123**:156-60.
- Barnes PJ, Wilson NM, Brown MJ. A calcium antagonist, nifedipine, modifies exercise-induced asthma. *Thorax* 1981;**36**:726-30.
- Patel KR. Calcium antagonists in exercise-induced asthma. *Br Med J*; **282**:932-3.
- Williams DO, Barnes PJ, Vickers HP, Rudolf M. Effect of nifedipine on bronchomotor tone and histamine



- reactivity in asthma. *Br Med J* 1981;**283**:348.
- <sup>26</sup> Henderson AF, Heaton RW, Costello JF. The effect of nifedipine in bronchoconstriction induced by inhalation of cold air. *Thorax* 1983;**38**:506–11.
- <sup>27</sup> Malik S, O'Reilly J, Sudlow MF. Effects of sublingual nifedipine on inhaled histamine and methacholine-induced bronchoconstriction in atopic subjects. *Thorax* 1982;**32**:230 (abstract).
- <sup>28</sup> Rolla G, Bucca C, Polizzi S, Maina A, Giachino O, -Salvini P. Nifedipine inhibits deep inspiration-induced bronchoconstriction in asthmatics. *Lancet* 1982;i:1305–6.
- <sup>29</sup> Patel KR, Al-Shamma M. Effect of nifedipine on histamine reactivity in asthma. *Br Med J* 1982;**284**:1916.
- <sup>30</sup> Jaiprakash SS, Sahay JN, Chatterjee SS, MacDonald G. Efficacy of nifedipine in the treatment of angina pectoris and chronic airways obstruction. *Postgraduate Med J* 1980;**56**:624–8.
- <sup>31</sup> Mongar JL, Schild HO. The effect of calcium and pH on the anaphylactic reaction. *J Physiol* 1958;**140**:272–86.
- <sup>32</sup> Foreman JC, Hallet MB, Mongar JL. The relationship between histamine secretion and <sup>45</sup>Ca uptake by mast cells. *J Physiol* 1977;**271**:193–214.
- <sup>33</sup> Ishizaka T, Hirata F, Ishizaka K, Axelrod J. Stimulation of phospholipid methylation Ca<sup>2+</sup> influx and histamine release by bridging of IgE receptors on rat mast cells. *Proc Natl Acad Sci USA* 1980;**77**:1903–10.
- <sup>34</sup> Foreman JC, Mongar JL. The action of lanthanum and manganese on anaphylactic histamine secretion. *Br J Pharmacol* 1973;**48**:527–33.
- <sup>35</sup> Suzuki T, Mori K, Uchida M. Inhibition by calcium antagonists of histamine release and calcium influx of rat mast cells: differences between induction of histamine release by concanavalin A and compound 48/80. *Eur J Pharmacol* 1982;**85**:155–61.
- <sup>36</sup> Agaki K, Tanizaji Y, Sano Y, Bewtra A, Townley R. Inhibitory effects of nifedipine on allergic reactions. *Clin Res* 1918;**29**:688A (abstract).
- <sup>37</sup> Hutchcroft BJ, Orange RP. The role of calcium in the generation of intracellular SRS-A in the passively sensitized human lung challenged with antigen. *Clin Allergy* 1980;**10**:565–73.
- <sup>38</sup> Cerrina J, Hadji L, Marche E, Duroux P, Benveniste J. Effect of the Ca<sup>2+</sup> antagonist nifedipine on histamine and SRS release from human lung tissue. *Am Rev Respir Dis* 1982;**125**:64 (abstract).
- <sup>39</sup> Lee VY, Hughes JM, Seale JP, Temple DM. Verapamil inhibits mediator release from human lung in vitro. *Thorax* 1983;**38**:386–7.
- <sup>40</sup> Cerrina J, Jouvin E, Duroux P, Benveniste J. Inhibition of the release of platelet activating factor and slow-reacting substance from human neutrophils by the calcium antagonist nifedipine. *Am Rev Respir Dis* 1981;**123**:44 (abstract).
- <sup>41</sup> Marin MG, Estep JA, Zorn P. Effect of calcium on sulfated mucous glycoprotein secretion in dog trachea. *J Appl Physiol* 1982;**52**:198–205.
- <sup>42</sup> Simonneau G, Escourrou P, Duroux P, Lockhart A. Inhibition of hypoxic pulmonary vasoconstriction by nifedipine. *N Engl J Med* 1981;**304**:1582–5.
- <sup>43</sup> Himori N, Taira N. Differential effects of the calcium antagonist vasodilators nifedipine and verapamil on the tracheal musculature of the dog. *Br J Pharmacol* 1980;**68**:595–7.
- <sup>44</sup> Becker EL, Stossel TP. Chemotaxis. *Fed Proc* 1980;**39**:2949–52.
- <sup>45</sup> Weishaar HE, Quade M, Kaplan HR. The methylene dioxindenes: a novel class of "intracellular calcium antagonists": their effect on processes involved in regulating calcium movement within the cell. *Fed Proc* 1982;**41**:1631 (abstract).
- <sup>46</sup> Levin RM, Weiss B. Selective binding of antipsychotic and other psychoactive agents to the calcium-dependent activation of cyclic nucleotide phosphodiesterase. *J Pharmacol Exp Ther* 1979;**208**:455–61.