concentrations (1 µg/0.1 ml and 10 µg/0.1 ml). Groups of animals were given 1 of these doses into the same injection site as PbAc 1 h before, at the same time as, 1, 2, 3, 4, 5, 6, and 8 h after the PbAc.

In the 4th experiment the effect of the combined administration of DSCG and cytochalasin A was tested in 72 mice divided into 18 groups of 4. Each animal was injected with PbAc 100 µg/0.1 ml into the lumbar region. Groups of animals were injected with both cytochalasin A (s.c. into the same site as PbAc) and DSCG (by the i.p. route) in the dose combinations 1 µg/0.1 ml s.c. and 0.3 mg/0.1 i.p. or 10 µg/0.1 ml s.c. and 3 mg/0.1 ml i.p. These combinations were injected 1 h before, at the same time as, 1, 2, 3, 4, 5, 6, and 8 h after the injection of PbAc.

In each experiment the s.c. injection sites were recovered after 7 days. Each site was X-rayed with a Faxitron cabinet X-ray machine and studied histologically and histochemically. The extent of the calcific reaction was determined by the chloranilic acid staining reaction for calcium (Eisenstein et al.[2]) and the von Kossa technique for phosphate.

Results. The injection of PbAc 100 µg/0.1 ml resulted in calcification in the control animals. This was macroscopically visible after 7 days and confirmed radiologically and histochemically. Animals which had been injected at the same time with DSCG (in either dose) showed identical areas of calcification. None of the drug treatments had any effect on development of local calcergy. Calcification occurred which was identical in extent and distribution to that found in the control animals.

Discussion. It has been shown that substances are released from rodent mast cells by 2 mechanisms one being a nonselective release of amine as a result of lysis of the cell membrane which is blocked by DSCG (Orr and Cox[3], Orr et al.[4], Orr et al.[5]) and the other a selective release of granules through a process of exocytosis (Ellis et al.[6]) which is mediated by microfilaments and blocked by cytochalasins (Orr et al.[7]). These separate mechanisms were blocked separately and in combination by the drug treatments used in the present experiments and none of these influenced the calcification reaction induced by PbAc and the reactions were indistinguishable from those in animals which had been injected with PbAc only.

In their study of local calcergy in the rat, Selye et al.[8] had suggested that discharged mast cell granules attracted lead, calcium and phosphate and that the resulting complex acted as a nidus which initiated a calcification reaction. Bridges and McClure[1] were unable to find any evidence in their study of local calcergy in the mouse to support this theory and instead suggested that mast cell degranulation played a nonessential role in the reaction being only associated with vascular dilatation and increased permeability.

In the present experiments the prevention of mast cell degranulation did not affect the calcergic reaction and therefore these results support the suggestion by Bridges and McClure[1] that the mast cell does not play an essential role in the pathogenesis of local calcergy in the mouse.

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Influence of clonidine on experimental hypertension induced by cholinergic stimulation

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Summary. Hypertension may be induced by pharmacologic activation of central cholinergic receptors either indirectly, through the injection i.v. of physostigmine, or directly, through the injection i.v. of arecholine in anesthetized rats. Activation of peripheral preganglionic cholinergic receptors with dimethylphenylpiperazinium iodide (DMPP) also produced a hypertensive response. Pretreatment with various doses of clonidine caused inhibition of the pressor response to central cholinergic stimulation but was without effect on the response to ganglionic cholinergic stimulation.

Peripheral[1] or central[2] injection of physostigmine elicits a rise in arterial blood pressure (BP), and this response is mediated through the release of endogenous brain acetylcholine (ACh) acting on muscarinic receptors. Centrally acting muscarinic receptor agonists, such as oxotremorine or arecholine, can activate this central cholinergic pathway by directly stimulating muscarinic receptors[3,4]. This elevation in BP is due to an increase in sympathetic nerve activity following central cholinergic stimulation[2,5]. We have recently reported[6] that administration i.v. of 100 µg of clonidine blocks the release of ACh from certain brain regions and as a result produces marked inhibition of the pressor response produced by injection i.v. of several doses of physostigmine. The present study was undertaken to elucidate the nature of the interaction between clonidine and the function of central and peripheral cholinergic neurons in the regulation of BP, and to determine whether this model of central cholinergic hypertension could be used to evaluate new antihypertensive drugs.

Male, Wistar rats weighing 250–400 g were anesthetized with urethane (1.3 g/kg, i.p.). Heparinized saline-filled polyethylene catheters were inserted into the left common carotid artery and left jugular vein for the direct recording of BP and injection i.v. of drugs, respectively. Drugs were dissolved in saline and injected in a volume of 0.1 ml/100 g b.wt. All drug weights refer to the salt. The basal level of mean arterial pressure (MAP) in 60 anesthetized rats averaged 74±2 mm Hg (mean ±SEM). Rats were initially injected with a control dose of cholinergic agonist, and after return of BP to preinjection levels, animals received clonidine. 30 min later a 2nd dose of agonist was administered. Preliminary experiments showed that all responses to cholinergic agonists were reproducible after consecutive injections spaced 30 min apart. When arecholine was used,

rats were pretreated with atropine methylbromide (1 mg/kg, i.v.). This quarternary derivative of atropine produced a selective blockade of peripheral muscarinic receptors and this prevented the marked hypotensive effect of arecholine due to its peripheral muscarinic action while allowing full expression of its hypertensive response due to central muscarinic stimulation.

Injection of physostigmine sulfate (50 µg/kg, i.v.) produced a maximum increase in MAP of 51±6 mm Hg. After return of BP to basal levels, the injection of clonidine (1, 10, 100 µg/kg, i.v.) produced an immediate, dose-related increase in MAP (10, 17 and 61 mm Hg, respectively). After BP was allowed to return to basal levels following clonidine, the animals were rechallenged with physostigmine. At doses of 10 and 100 µg/kg, clonidine reduced the pressor response to physostigmine by 57% and 82% (figure 1).

To further localize the site of action of clonidine, the ganglionic stimulant, dimethylphenylpiperazinium iodide (DMPP), was used to activate peripheral sympathetic neurons. Rapid injection i.v. of DMPP (100 µg/kg) produced a maximum increase in MAP of 40±8 mm Hg (figure 2). Clonidine pretreatment, however, failed to inhibit the pressor response to ganglionic stimulation.

To determine whether clonidine's ability to block the pressor effect of physostigmine was the result of inhibition of ACh release in the CNS or sympathetic ganglia, arecholine hydrochloride was used. Under conditions of peripheral muscarinic blockade, arecholine, like physostigmine, evoked a hypertensive response which is mediated through central muscarinic receptors. Unlike physostigmine, however, the pressor response to arecholine is mediated independently of brain ACh release. Injection of arecholine (50–500 µg/kg, i.v.) evoked a dose-related increase in MAP of 30–73 mm Hg (figure 3). In experiments where rats received 10 µg/kg of clonidine (a dose which reduced the pressor response physostigmine by 57%) there was no alteration of the pressor response to a subsequent injection of arecholine. In contrast, the 100 µg/kg dose of clonidine shifted the dose-pressor response curve approximately 5-fold to the right. This inhibition by the highest dose of clonidine might also account for its effect on the physostigmine pressor response.

High concentrations of clonidine have previously been reported to inhibit ganglionic transmission[7], although this effect is not generally considered to be important when clinically relevant doses are used to lower BP. Our results

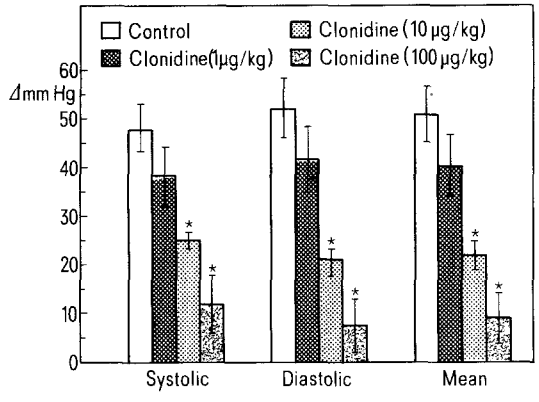

Fig. 1. Effect of several i.v. doses of clonidine on the maximum increase in blood pressure produced by physostigmine sulfate (50 µg/kg). * = significantly different ($p < 0.05$) from control value (Student's t-test). Each mean is the average of 5 experiments.

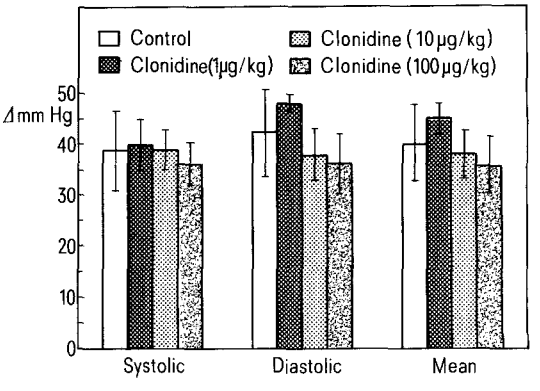

Fig. 2. Effect of several i.v. doses of clonidine on the maximum increase in blood pressure produced by DMPP (100 µg/kg). Each mean is the average of 5 experiments.

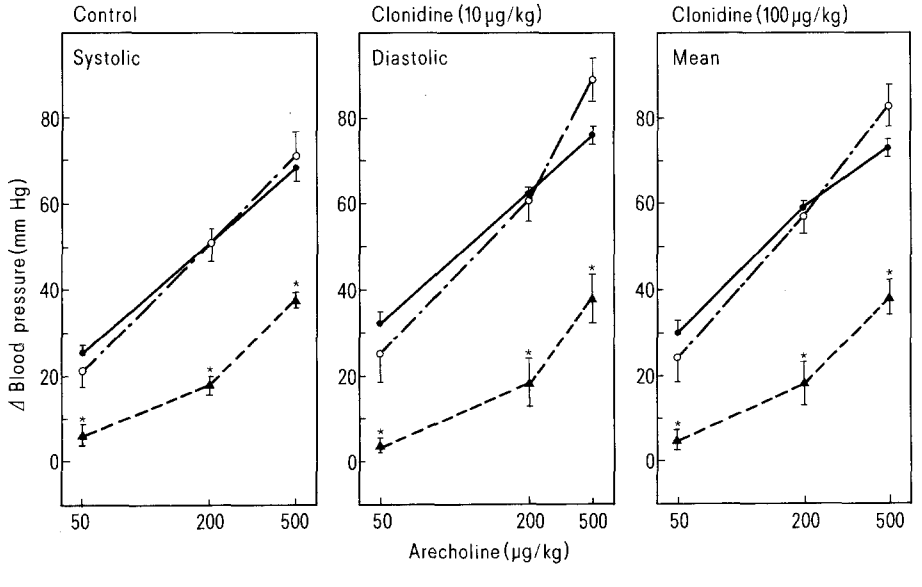

Fig. 3. Effect of 10 or 100 µg/kg of clonidine on the dose-pressor response relationship produced by i.v. injection of arecholine. * = significantly different from control values. Each mean is the average of 5 experiments.

support these findings since the ability of clonidine to block the pressor response to arecholine at the highest dose is most likely due to blockade of ganglionic transmission. Clonidine, by acting at α-adrenergic receptors located on peripheral cholinergic nerve terminals can block the release of ACh[8,9]. Alternatively, clonidine could also block ganglionic transmission by occupying pre-ganglionic nicotinic receptors. The lack of effect of clonidine on the pressor response to DMPP, however, rules out this possibility. Also, the fact that the 10 µg/kg dose of clonidine blocked the pressor response to physostigmine but not that to arecholine indicates that central cholinergic neurons are more susceptible to the inhibitory effects of clonidine than are peripheral cholinergic neurons. This difference may be related to the actual concentration of clonidine present at central or peripheral sites following injection i.v. of the drug and/or the density of α-adrenergic receptors located on the nerve terminals of these cholinergic neurons.

The induction of hypertension by a central cholinergic mechanism offers another animal model to evaluate the mechanism of action of many antihypertensive drugs, and also provides another approach to the understanding of human essential hypertension.

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The role of hyperoxygenation in facilitating the induction of pulmonary histiocytosis by low doses of chlorphentermine[1]

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Summary. Hyperoxygenation modified the susceptibility of neonatal rat lung to respond to chlorphentermine with an accumulation of hypertrophic macrophages, a morphologic change undetected when an anorectic drug was given alone. Implications of this observation for pediatrics are discussed.

In adult rats, treatment with 20 mg/kg chlorphentermine for 8 days produced an accumulation of masses of foam cells (hypertrophic, phospholipid-rich histiocytes) in the alveoli and bronchi of the lungs[4]. In contrast, Kacew et al.[5] found that the daily administration of 20 mg/kg chlorphentermine for 1 week failed to produce any apparent morphologic alteration in neonatal rat lung. However, an increase in anorectic drug dose to 40 or 60 mg/kg resulted in an accumulation of hypertrophic macrophages in newborn pulmonary alveoli, suggesting that neonates may be less responsive to chlorphentermine than adults. In view of the fact that neonates are also less susceptible to the toxic actions of high concentrations of oxygen[6] and oxygen is known to modify drug-induced lung changes[7] it was of interest to examine the interaction between chlorphentermine and hyperoxygenation on pulmonary morphology.

Methods. Female rats of the Sprague-Dawley strain with 1 day-old litters were used. Chlorphentermine (20 mg/kg) was administered daily by gastric intubation. Corresponding controls received an equal volume (50 µl) of physiological saline. For hyperoxia experiments, animals were maintained for 3 days in a spherical airtight plexiglass chamber filled with 95% O_2 at a rate of 3 l/min and gas was monitored with a Beckman model OM-11 gas analyzer. In sequential experiments, pups which had previously received 20 mg/kg/day chlorphentermine for 6 days were subsequently exposed to 95% O_2 for 72 h, or newborns which were initially exposed to 95% O_2 for 3 days were given the drug for 6 days. In addition, groups of newborns were simultaneously exposed to 95% O_2 and given chlorphentermine for either 1, 2, 3 or 5 days. From each animal, portions of one lung were fixed and processed for electron microscopy as described previously[8], and the contralateral lung was fixed intact in Bouin's fixative and processed for light microscopy. Although few alveolar macrophages were found both in control and experimental animals, only the hypertrophic, vacuolized macrophages ('foam cells') were counted. Quantitation of foam cells (FC) in alveoli was conducted using an arbitrary scale as follows: (+ + +), 1-4 FC in many peripheral alveoli of all experimental animals; (+ +), 1-2 FC in few peripheral alveoli of all experimental animals; (+), 1-2 FC in few peripheral alveoli of at least half the experimental animals; (−), no FC observed.

Results. The lung and body weights of control rats used in this study were approximately 200 mg and 7 g, respectively. In all treated groups lung and body weights did not differ significantly from those of the respective controls throughout the experimental period. In cases where animals failed

Effect of hyperoxygenation and chlorphentermine on pulmonary foam cell reaction in newborn rats

Series number	Number of animals	Treatment schedule	Foam cell quantitation*
1	4	Control	−
2	4	O_2 alone for 3 days	−
3	10	Drug alone for 6 days	−
4	8	O_2 for 3 days followed by drug for 6 days	+ + +
5	8	Drug for 6 days followed by O_2 for 3 days	+
6	4	Simultaneous drug and O_2 for 1 day	+
7	4	Simultaneous drug and O_2 for 2 days	+ +
8	4	Simultaneous drug and O_2 for 3 days	+ + +
9	4	Simultaneous drug and O_2 for 5 days	+ +

* See materials and methods.