PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 28-33 (1976)

Differential Effects of *p*-Chloromercuriphenyl Sulfonate on Arterial Responses to Epinephrine and Serotonin[1] (39474)

JOSEPH DISALVO

Department of Physiology and Department of Medicine, College of Medicine, University of Cincinnati, Cincinnati, Ohio 45267

The purpose of this investigation was to study the effects of *p*-chloromercuriphenyl sulfonate (PCMBS), a potent sulfhydryl (SH) binding agent (1-6), on contractile responses elicited with epinephrine and serotonin in isolated preparations of arterial smooth muscle. In this way, we could examine whether or not SH groups are importantly involved in agonist-receptor systems for vasoactive stimulants.

It is generally stated that vascular receptor-sites for vasoactive agents such as acetylcholine, epinephrine, and serotonin consist largely of proteins (7-9). The importance of SH groups in maintaining biological activity has been well established for a variety of protein structures including enzymes (10), membrane-bound transport moieties (1-4), and steroidal (5) and cholinergic receptors (6). However, the extent to which SH groups represent important functional groups of receptors for epinephrine or serotonin in arterial smooth muscle is unknown. Such information is of interest because chemical characterization of vasoactive receptor-sites could provide insight into the mechanistic basis of agonist-receptor interactions.

Methods. Sprague-Dawley rats of either sex (350-450 g) were anesthetized with ether and decapitated. Helical strips of arterial smooth muscle measuring about 4 to 6 mm long, 400 μm wide and 1 mm thick were prepared from the ventral tail artery as described previously (11).

Four arterial strips (two from each of two rats) were mounted in a single muscle chamber. One end of each strip was fixed to a separate post while its other end was attached to an isometric force transducer (Grass, FT-03) by means of a silk thread. The strips were stretched to optimal length by imposing a resting tension of 500 mg and equilibrated for 2 hr in 20 ml of physiological salt solution (PSS) which was maintained at 37°C and gassed continuously with 95% O_2 and 5% CO_2 (11). The pH of the PSS was 7.25 to 7.35, and its composition (mmole) was NaCl 130, KCl 4.7, KH_2PO_4 1.18, $MgSO_4 \cdot 7H_2O$ 1.17, $CaCl_2$ 1.6, $NaHCO_3$ 14.9, $CaNa_2$ versenate 0.026, and dextrose 5.5.

Serotonin creatinine sulfate (Mann Labs) or the racemate mixture of epinephrine-HCl (Parke-Davis) was dissolved in PSS and added (0.2 ml) to the tissue bath. The final concentration (10^{-5} *M*) of each agonist tested produced maximal increases in isometric tension. However, the concentration of serotonin represents a maximal dose, whereas the concentration of epinephrine was supramaximal (11).

Responsiveness of the muscle strips to several challenges with epinephrine and with serotonin was examined at the beginning of each experiment. The maximal tension generated during a 2 min exposure to the agonist tested was designated as the control response. After each challenge with agonist, the muscle chamber was rinsed three times with PSS so that tension returned to baseline levels.

The bathing medium was replaced with PSS supplemented with PCMBS (Sigma, 10^{-6} to 10^{-4} *M*). Only one concentration of PCMBS was examined in each experiment. Challenges with agonists were tested after about 10, 30, 60, and 120 min of contact with PCMBS. Preliminary experiments performed in the absence of PCMBS showed that repeated responses to either serotonin or epinephrine were reproducible over this period of incubation. When the last challenge was completed, the chamber was

[1] This work was supported by a grant from the Southwestern Ohio Chapter of the American Heart Association and by a Government Research Support Grant, RR5408-14 (No. 13).

rinsed at 10 min intervals with PCMBS-free PSS and the strips were again challenged to test for recovery of responsiveness. Contractile responses obtained in the presence of PCMBS, as well as those obtained during the recovery period, were expressed as a percentage of the control response. Statistical significance of differences between responses produced by each agonist in the presence and absence of PCMBS was assessed with Student's t test.

To determine whether or not cysteine, an amino acid containing an SH group, protects against inhibition of responses to epinephrine, cysteine (10^{-3} M) and PCMBS (10^{-5} M) were added to the muscle chamber at the same time in three different experiments (12 strips).

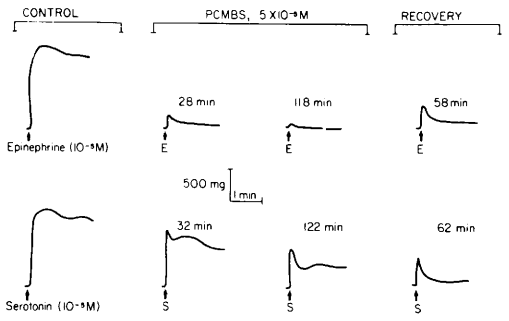

FIG. 1. Contractile responses to epinephrine and serotonin in a single arterial strip are shown before, during incubation with PCMBS, and after replacing the medium with PCMBS-free PSS. Arrows indicate time of injection of agonist into the muscle chamber. Note that the response to serotonin was maintained moderately (28 min of contact with PCMBS) while the response to epinephrine was markedly inhibited.

The effects of alpha-adrenergic blockade with 10^{-4} M phentolamine (Ciba) on responsiveness to epinephrine and serotonin was examined in eight different tail artery strips prepared from four rats. Responses were evaluated before and 30 min after incubation with phentolamine. Similar experiments were performed with six additional preparations (three rats) in the presence and absence of 5×10^{-6} M phentolamine.

To test for the presence of beta-adrenergic receptors, responses to DL-isoproterenol (MCB, 10^{-9} to 10^{-5} M) were evaluated in eight preparations from four rats in the presence and absence of phentolamine (5×10^{-6} M).

Results. Maximal isometric tension generated by rat tail artery smooth muscle in response to 10^{-5} M epinephrine (1341 ± 104 mg) was similar to the tension generated in response to 10^{-5} serotonin (1296 ± 107 mg).

PCMBS consistently produced time and dose-dependent inhibition of contractile responses elicited with either epinephrine or serotonin (Figs. 1, 3, Table I). In other words, inhibition of responsiveness became progressively more pronounced as the duration (10–120 min) of incubation with each concentration of PCMBS was lengthened. Similarly, the extent of inhibition attained after a given period of incubation with PCMBS increased markedly as the concentration (10^{-6} to 10^{-4} M) of the sulfhydryl binding agent was increased.

However, inhibition of responses to epinephrine always was dramatically more pro-

TABLE 1. CONTRACTILE RESPONSES TO EPINEPHRINE AND SEROTONIN IN THE PRESENCE OF PCMBS.[a]

Agonist	Time of PCMBS (min)	Contractile response (% of control)		
		Concentration of PCMBS (M)		
		10^{-6}	7.5×10^{-6}	5×10^{-5}
Epinephrine (10^{-5} M)	10	96 ± 4	71 ± 2	1 ± 0.4
	30	94 ± 4	37 ± 4	0.7 ± 0.4
	60	90 ± 3	9 ± 5	0
	120	82 ± 4	2 ± 1	0
Serotonin (10^{-5} M)	10	96 ± 2	82 ± 2	73 ± 3
	30	92 ± 4	76 ± 2	75 ± 4
	60	89 ± 5	70 ± 3	58 ± 4
	120	88 ± 2	62 ± 4	60 ± 2

[a] Eight helical strips of tail artery smooth muscle (four rats) were studied with each concentration of PCMBS (see Methods). Numbers appearing below each concentration of PCMBS show the response (% of control) to epinephrine or serotonin (mean ± 1 SE). PCMBS markedly inhibited responses to epinephrine but responses to serotonin were depressed to a smaller extent.

nounced than the inhibition of responses to serotonin. Thus, the contractile response to epinephrine was markedly reduced to 41 ± 2% of control (59% inhibition, Fig. 2B) after a 10 min exposure to an intermediate concentration (10^{-5} *M*) of PCMBS, whereas the response to serotonin was only decreased to 86 ± 2% of control (14% inhibition, $P < 0.001$). Furthermore, the response to epinephrine was virtually abolished after 120 min of incubation with PCMBS while the response to serotonin had decreased by only about 50% ($P < 0.001$). Indeed, complete inhibition of epinephrine-induced contractions was achieved regularly with concentrations of PCMBS ranging from 5×10^{-6} *M* to 10^{-4} *M* (Fig. 2, Table 1). In contrast, serotonin-induced contractions were always demonstrable at each concentration of PCMBS tested.

Considerable, but incomplete, recovery of responsiveness to epinephrine occurred when the bathing medium containing PCMBS was replaced with PCMBS-free PSS (Fig. 3A). The extent of recovery was inversely related to the concentration of PCMBS initially tested. In sharp contrast, responsiveness to serotonin either was unchanged or deteriorated further when PCMBS-free PSS was returned to the muscle chamber (Fig. 3B).

PCMBS-induced inhibition of responsiveness to epinephrine was prevented when cysteine (10^{-3} *M*), an SH containing amino acid, was added to the bathing medium just before addition of 10^{-5} *M* PCMBS. Thus, the tension generated in response to epinephrine was 1463 ± 133 mg in 12 strips studied in the absence of PCMBS, and 1413 ± 107 mg ($P < 0.1$) after 120 min of contact with 10^{-5} *M* PCMBS and 10^{-3} cysteine.

Alpha-adrenergic blockade with 10^{-4} *M* phentolamine virtually abolished the contractile response to epinephrine. In eight strips tested, tension produced by epinephrine was 1348 ± 112 mg in the control setting and 108 ± 74 mg ($P < 0.001$) 30 min after incubation with phentolamine. Responses to serotonin were significantly ($P < 0.005$) reduced during alpha-blockade. Before blockade, responses to serotonin generated 1236 ± 107 mg of tension, but only 717 ± 44 mg was generated in the

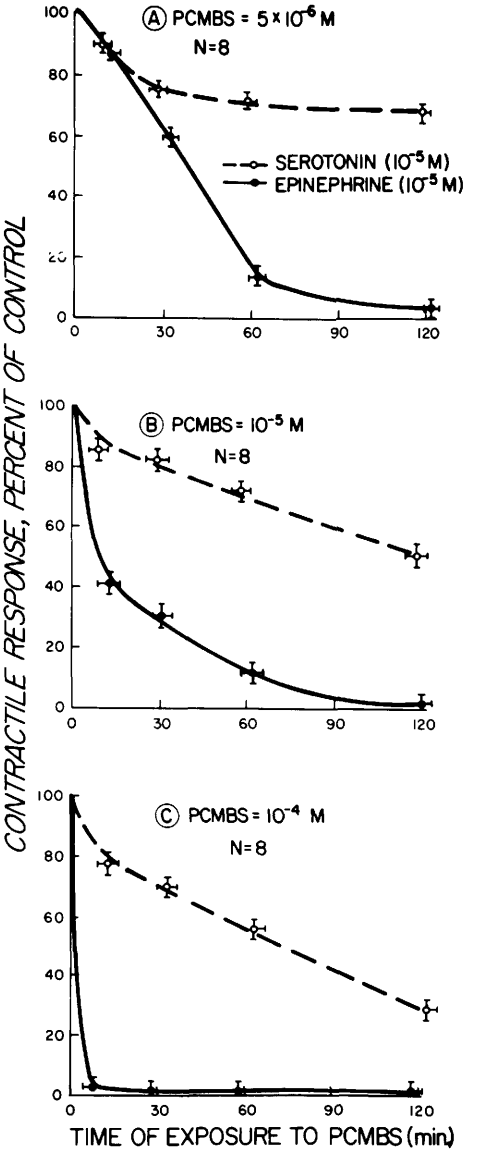

FIG. 2. Effects of different concentrations of PCMBS on arterial responsiveness to epinephrine and serotonin. Different arterial strips were used for data shown in each panel. Small verticle bars represent 1 SE of the mean response (% of control), whereas horizontal bars represent 1 SE of the mean duration of contact (min) with PCMBS. Inhibition of responses to epinephrine was always more rapid in onset and more pronounced than inhibition of responses to serotonin.

presence of phentolamine. Similar results were obtained with 5×10^{-6} *M* phentolamine in eight different arterial preparations. In these strips, epinephrine increased tension to 1487 ± 136 mg before treatment

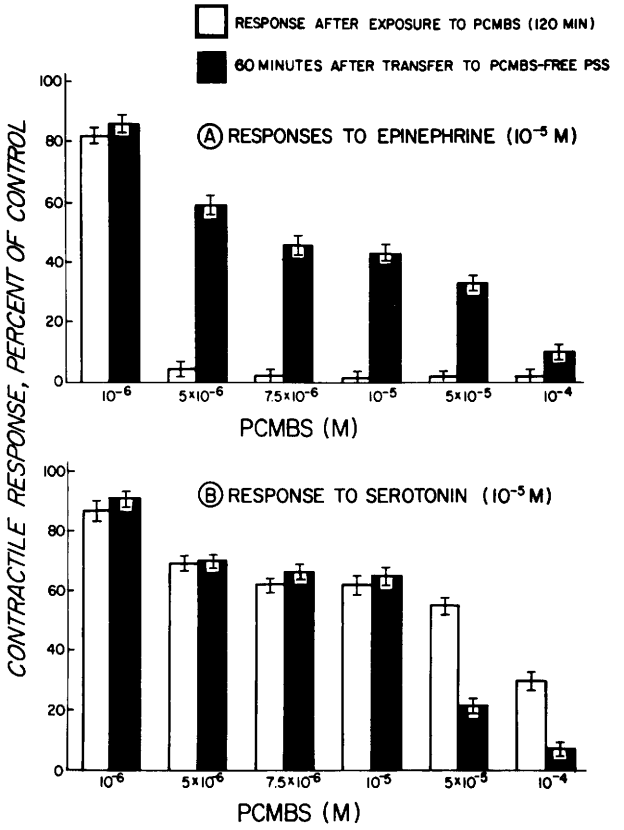

FIG. 3. Responsiveness of arterial strips to epinephrine (panel A) and serotonin (panel B) 120 min after incubation with different concentrations of PCMBS (clear bars), and 60 min after replacing the medium with PCMBS-free PSS (dark bars). Small verticle bars represent ± 1 SE. Eight different arterial strips were tested at each concentration of PCMBS. Responsiveness to epinephrine recovered markedly after PCMBS was removed from the muscle chamber, but responsiveness to serotonin did not recover.

with phentolamine, whereas only 196 ± 88 mg ($P < 0.001$) of tension were generated in the presence of phentolamine. Similarly, tension generated in response to serotonin was 1314 ± 100 mg before blockade, but only 982 ± 44 mg ($P < 0.005$) of tension developed after blockade with 5×10^{-6} *M* phentolamine.

Low doses of isoproterenol (10^{-9} to 10^{-7} *M*) produced no change in tension (8 strips). However, higher doses produced small dose-dependent increases in isometric tension. For example, 10^{-6} *M* isoproterenol increased tension to 57 ± 9 mg while tension increased to 136 ± 14 mg with 10^{-5} *M* isoproterenol. These contractile responses were completely abolished after alpha-adrenergic blockade with 5×10^{-6} *M* phentolamine.

Discussion. A prominent new finding in this study was that short periods of incubation (10–30 min) in the presence of low doses of PCMBS (10^{-6} to 10^{-5} *M*) markedly inhibited contractile responses to epinephrine in rat tail arterial smooth muscle. In contrast, longer periods of incubation (60–90 min) and higher doses of PCMBS (10^{-5} to 10^{-4} *M*) were required to markedly inhibit responses to serotonin (Figs. 1–3, Table I). These new findings suggest that SH groups may be important functional components of alpha-adrenergic receptors in arterial smooth muscle.

However, because epinephrine can stimulate both alpha and beta-adrenergic receptors, it is also conceivable that depression of responses to epinephrine with PCMBS is ascribable to potentiation of interactions

between epinephrine and beta-adrenergic receptors. This possibility does not appear likely because isoproterenol, though generally described as a beta-adrenergic stimulating agent (12), produced small increases in isometric tension. Furthermore, isoproterenol-induced contractions were blocked by phentolamine, an observation consistent with previous findings showing that isoproterenol can stimulate alpha-adrenergic receptors (13, 14). Accordingly, the inhibitory effects of PCMBS on contractile responses to epinephrine in tail arterial smooth muscle is likely due to interactions between PCMBS and SH groups associated with alpha-adrenergic receptors.

In contrast, responses to stimulation of serotonin receptors appear to be much less dependent on SH groups. An alternative hypothesis to account for the persistence of serotonin responses is that the accessibility of SH groups that might be associated with serotonin receptors is restricted so that their interaction with PCMBS is hindered.

Our interpretation of the data is based on studies in other laboratories which established that PCMBS is a potent and highly selective SH binding agent and that it is poorly permeant in a variety of cell types (1-6). In this context, our finding that cysteine, an amino acid with an SH group, protected arterial strips against PCMBS-induced inhibition of responsiveness to epinephrine suggests that the ability of PCMBS to bind SH groups is an important determinant of the efficacy of this compound to inhibit adrenergic responses. Furthermore, the rapidity of the onset of inhibition (Fig. 1) is consonant with the view that the inhibitory effect of PCMBS is mediated at sites close to the exterior of the smooth muscle cell, perhaps at the level of the plasma membrane. This view is also supported by the observation that marked and rapid recovery of responsiveness to epinephrine occurred when the bathing medium was replaced with PCMBS-free PSS (Fig. 3A).

Surprisingly, recovery of responsiveness to serotonin did not occur when PCMBS was washed from the muscle chamber (Fig. 3B). Thus, at each dose of PCMBS tested, responses to epinephrine were consistently more depressed than were responses to serotonin, but the fractional recovery of responsiveness was always greater with epinephrine. The mechanistic basis for this interesting observation is unknown. Nevertheless, it is noteworthy that recovery of responsiveness for either epinephrine or serotonin was always incomplete, suggesting that a fraction of the PCMBS initially included in the incubation medium was tightly, perhaps irreversibly, bound to the smooth muscle strips.

The present findings that alpha-adrenergic blockade with phentolamine abolished responses to epinephrine and significantly attentuated responses to serotonin are consistent with results obtained by other investigators studying human umbilical arteries and veins (15), rabbit portal vein (16), and canine saphenous, jugular, and mesenteric veins (17). These results suggest that vascular smooth muscle receptors for serotonin and epinephrine may be structurally related. It is tempting to speculate that such structural relationships could account for, or contribute to, the partial inhibition of serotonin-induced contractions by PCMBS (Fig. 1, Table I). Further studies are required to test this hypothesis.

We recognize that PCMBS may influence arterial responsiveness to agonists by mechanisms other than those involving interactions with SH groups of receptor moieties. However, our findings showing that the inhibitory effects of the compound were selective, time and dose-dependent, rapid in onset, partially reversible, and preventable by cysteine, strongly suggest that PCMBS interacts with SH groups present in arterial smooth muscle receptors for vasoactive stimulants.

Nevertheless, it is important to emphasize that different compounds which interfere with SH groups may produce different effects on the responsiveness of arterial smooth muscle to vasoactive stimulants. For example, Needleman *et al.* (18) reported that alkylation of SH groups with ethacrynic acid did not alter contractile responses elicited with norepinephrine in rabbit aortic strips. In contrast, Fleisch *et al.* (19) found that alkylation with *N*-ethylmaleimide significantly reduced responses to norepinephrine in similar preparations from rabbit aorta. The reasons for these disparite findings are unclear but may be related to differ-

ences between molecular size and charge density of the alkylating agents (1, 2). Our findings with rat tail arterial smooth muscle are fundamentally in accord with those reported by Fleisch *et al.* (19) in that high concentrations of *N*-ethylmalemide (10^{-4} *M*, rabbit aorta), like high concentrations of PCMBS (rat tail artery), inhibited responses to serotonin.

Summary. The purpose of this study was to gain insight into the role of sulfhydryl (SH) groups in arterial receptors for vasoactive simulants. We studied the effects of *p*-chloromercuriphenyl sulfonate (PCMBS, 10^{-6} to 10^{-4} *M*), a potent and specific SH binding agent, on contractile responses produced with 10^{-5} *M* epinephrine and 10^{-5} *M* serotonin in helical strips of arterial smooth muscle prepared from tail arteries of the rat. Responses to epinephrine were rapidly, markedly, and reversibly inhibited with low concentrations of PCMBS. In contrast, responses to serotonin were slowly inhibited when high concentrations of PCMBS were tested. The inhibitory effect of PCMBS (10^{-5} *M*) on epinephrine-induced contractions was prevented by 10^{-3} *M* cysteine. Alpha-adrenergic blockade with phentolamine (10^{-4} or 5×10^{-6} *M*) virtually abolished responses to epinephrine and significantly decreased responses to serotonin. These findings are consistent with the view that arterial receptors for contractile responses to epinephrine and serotonin may be structurally related. The data suggest that SH groups are important functional components of adrenergic receptors, but that they are of lesser importance for responsiveness of serotonin receptors.

1. Vanstevenick, J., Weed, R. I., and Rothstein, A., J. Gen. Physiol. **48,** 617 (1965).
2. Rothstein, A., *In* "Current Topics in Membranes and Transport," vol. 1, p. 35. Academic Press, New York (1970).
3. Ullrich, K. J., Fasold, H., Kloss, S., Rumrich, G., Salzer, M., Sato, K., Simon, B., and de Vries, J. X., Pflügers Arch. **334,** 51 (1973).
4. Schaeffer, J. F., Preston, R. L., and Curran, P. F., J. Gen. Physiol. **62,** 131 (1973).
5. Karlin, A., and Bartels, E., Biochem. Biophys. Acta **126,** 525 (1966).
6. Levinson, B. B., Baster, J. D., Rousseau, G. G., and Tomkins, G. M., Science **175,** 189 (1971).
7. Furchgott, R. F., Ann. Rev. Pharmacol. **4,** 21 (1964).
8. Waud, D. R., Pharmacol. Rev. **20,** 49 (1968).
9. Goldstein, A., Aronow, L., and Kalman, S. M., "Principles of Drug Action," p. 1. John Wiley & Sons, New York (1974).
10. Skou, J. C., and Hiberg, C., Biochim. Biophys. Acta **110,** 359 (1965).
11. DiSalvo, J., and Schmidt, C., Proc. Soc. Expt. Biol. Med. **151,** 478 (1976).
12. Ahlquist, R. P., *In* "Handbook of Physiology Circulation" (W. F. Hamilton and P. Daro, eds.), Sect. 2, vol. 3, p. 2457. American Physiological Society, Washington, D.C. (1965).
13. Mark, A. L., Eckstein, J. W., Abboud, F. M., and Wendling, M. G., Amer. J. Physiol. **217,** 764 (1969).
14. DiSalvo, J., Fell, C., Proc. Soc. Exp. Biol. Med. **133,** 1435 (1970).
15. Astrom, A., and Samelius, U., Brit. J. Pharm. **12,** 410 (1957).
16. Hughes, J., and Vane, J. R., Brit. J. Pharm. **30,** 46 (1967).
17. Clement, D., Vanhoutte, P., and Leusen, I. Arch. Int. Physio. Biochem. **77,** 73 (1969).
18. Needleman, P., Jakschik, B., and Johnson, E. M., J. Exp. Pharm. Ther. **187,** 324 (1973).
19. Fleisch, J. H., Krzan, M. C., and Titus, E., Circul. Res. **33,** 284 (1974).

Received December 18, 1975. P.S.E.B.M. 1976, Vol. 153.