TISSUE SENSITIVITY TO ACETYLCHOLINE, ADRENALINE, NORADRENALINE AND SEROTONIN AND AGENTS ACTING ON SULPHYDRYL GROUPS

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Summary : Parachloromercury benzoate (PCMB), a sulphydryl inactivator, caused a progressively increasing inhibition of tissue responses to acetylcholine, adrenaline, noradrenaline and serotonin *in vitro*. This inhibition was progressively and completely reversed by penicillamine, a sulphydryl activator. It is inferred that intact sulphydryl groups are essential for constancy of responses of excitable tissues to the neurotransmitters.

Key words : parachloromercury benzoate, penicillamine, sulphydryl, neurotransmitter, tissue sensitivity

INTRODUCTION

Sulphydryl agents are known to cause changes in cell membrane permeability by acting on membrane sulphydryl groups (1, 2, 7, 10). Such changes in permeability are bound to modify the activity of agonists at their receptor sites. Further, receptors for certain neurotransmitters, viz. acetylcholine, adrenaline, noradrenaline and serotonin are endowed with sulphydryl groups (3, 4, 6, 13). These groups undergo sulphydryl disulphydryl interconversion with the changes in the level of activity of the receptors and their effector cells (3, 4, 14). In view of this, the influence of two sulphydryl agents viz., parachloromercuribenzoate (PCMB), a sulphydryl inactivator, and penicillamine, a sulphydryl activator (1, 8) was studied on the sensitivity of excitable tissues to certain neurotransmitters.

MATERIAL AND METHOD

Four types of *in vitro* experiments were performed to test the effect of acetylcholine, adrenaline, noradrenaline and serotonin (Table I). Standard methods of dissection, excision and isolation of the tissues were adopted (9). The tissues were mounted in 25 *ml* organ bath in bathing media constantly bubbled with oxygen and maintained at 37°C. Isotonic lever was used to record the responses on the smoked kymograph drum. Each preparation was stabilised till it responded to the test-neurotransmitter reproducibly

and then a single test dose of the agonist (producing 50% of maximal response) was selected and its responses were recorded in the absence and in presence of the fixed concentration of either PCMB or penicillamine at intervals of 5–10 min.

PCMB 10 or 50 μmol/l and penicillamine 68 μmol/l were prepared in physiological solutions freshly before use.

RESULTS

From the Fig. 1, it is evident that acetylcholine-induced contraction of rectus abdominis decreased progressively in presence of 50 μmol/l of PCMB. The acetylcholine-response was suppressed to 1/5 in 50 min by PCMB. The PCMB inhibition of acetylcholine-induced contraction was completely reversed by penicillamine (68 μmol/l) in 40 min.

Noradrenaline-induced contraction of rat vas deferens was inhibited completely by PCMB (10 μmol/l) in 10 min and this inhibition was overcome by penicillamine (68 μmol/l) in 25 min.

TABLE I : Tissues empolyed to study effect of sulphydry reactants.

Neurotransmitter tested	*Animal*	*Tissue**	*Physiological solution*
1. Acetylcholine Chloride	Frog	Rectus abdominis	Ringer
2. (–) Adrenaline hydrochloride	Rabbit	Seminal vesicle	Tyrode
3. (–) Noradrenaline bitartrate	Rabbit	Vas deferens	Tyrode
4. 5-Hydroxytryptamine Creatinine sulfate	Rabbit	Uterus	De Jalon's

*5 Experiments were performed with each tissue.

Adrenaline-induced contraction of rabbit seminal vesicle was completely blocked by PCMB (50 μmol/l) in 20 min and the blockade was completely overcome by penicillamine in 25 min.

5-HT-induced contraction of rat uterus was completely suppressed by PCMB (50 μmol/l) in 25 min and this inhibition was completely reversed by penicillamine (68 μmol/l) in 25 min.

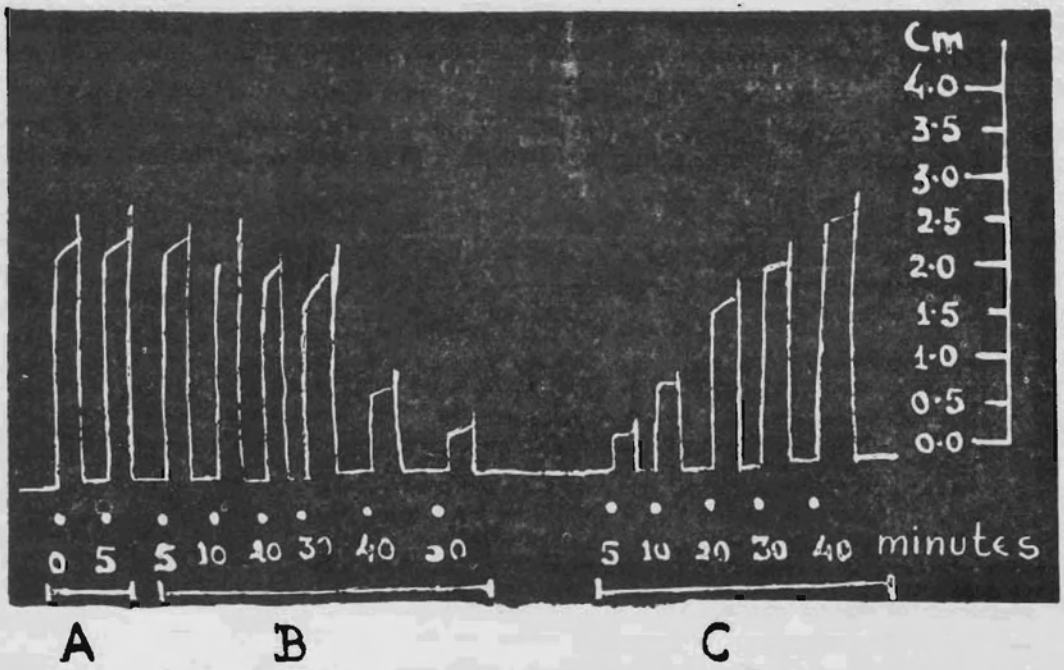

Fig. 1 : Frog rectus abdominis muscle. Contraction induced by acetylcholine (1.5 *µg/ml*) (at dots). A, control responses B. Responses in presence of PCMB (50 *µmol/l*). C. Responses in presence of penicillamine (68 *µmol/l*).

In all the experiments PCMB-induced inhibition of the responses of the neurotransmitters was not decreased even after 25–40 min of washing out the preparations with the respective physiological solutions. However, in this period of time complete reversal of the responses occured consistently, if penicillamine was present in the bathing solution.

DISCUSSION

PCMB-induced inhibition of tissue responses could have been due to decreased membrane permeability of the different neurotransmitter substances on to the receptor sites (2, 10, 12) and or due to the reduction in sensitivity (or blockade) of the receptor (3, 4, 6, 13). PCMB penetrates the tissues very slowly (8); the initial inhibition of tissue sensitivity to neurotranamitters might have been due to inactivation of sulphydryl groups located on the cell surface rather than these located deep in the cells.

In all experiments penicillamine antagonized the blockade of activity of the tested neurotransmitters induced by PCMB. Reversal of blockade by penicillamine was gradual and progressive and this is suggestive of reversal of sulphydryl inhibition by penicillamine

which may augment membrane permeability (7) to test-neurotransmitters, or of the receptor sensitivity to control levels. Further, release and transport of membrane-bound calcium is also influenced by the activity of the tissue sulphydryl groups. Calcium-sensitive SH-groups exist at a site which is essential for the regulatory function of mitochondria and sarcoplasmic reticulum (5, 9, 11). PCMB-inhibition and its reversal by penicillamine of the responses of tested neurotransmitters may also implicate sulphydryl dependent calcium mobility.

REFERENCES

1. Dand, E. and G. Leblanc. The effect of ethacrynic acid and other sulphydryl reagents on sodium fluxes in frog muscle. *J. Physiol. (London)*, **214** : 327–347, 1971.
2. Daniel, E.E., A.M. Kidwai. K. Robinson, O. Freeman and S. Fair. The mechanisms by which ethacrynic acid affects ion content, ion fluxes volume and energy supply in rat uterus. *J. Pharmac. Exp. Ther.*, **176** : 563–579, 1971.
3. Goldman J.M. and M.E. Hadley. Sulphudryl requirement for alpha adrenergic receptor activity and melanophore stimulating hormone (MSH) action on melanophores. *J. Pharmac. Exp. Ther.*, **182** : 93–100, 1972.
4. Harvey, S.C. and M. Nickerson. Reactions of Dibenamine and some congeners with substances of biological interest in relation to the mechanisms of adrenergic blockade. *J. Pharmac. Exp. Ther.*, **112** : 274–290, 1954.
5. Hasselbach, W. and K. Seraydarian. The role of sulphudryl groups in calcium transport through the sarcoplasmic membranees of skeletal muscle. *Biochem. Z.* **345** : 159–172, 1966.
6. Karlin, A. Molecular interactions of acetylcholine receptor *Fed.Proc.*, **32** : 1847, 1973.
7. Motais, R. and F. Sola Characterstics of a sulphydryl group essential for sodium exchange diffusion in beef erythrocytes. *J. Physiol. (London)*, **233** : 423–438, 1973.
8. Neering, I.R. and W.E. Glover. The role of sulphydryl groups in contraction of vasular smooth muscle. *J. Pharmac. Exp. Ther.*, **208** : 355–340, 1970.
9. Perry W.L.M. "Pharmacological experiments on isolated preparations by Staff of the Deptt. of Pharmacology, Second Edition, London : University of Eninburgh, 1970.
10. Scott, K.M., A.V. Knight, C.T. Settlemire and G.P. Brierly. Differential effects of mercurial agents on membrane thiols and on the permeability of the heart mitochondrion. *Biochemistry*, **9** : 714–724, 1970.
11. Tolbery, A.G. and R.I. Macey. The release of membrane bound calcium by radiation and sulphydryl reagents. *J. Cell. Physiol.*, **79** : 43–51, 1972.
12. Tongia, S.K. and K. Pandya. Modification of adrenaline Mydriasis by agents acting on tissues sulphydryl groups. *Ind. J. Physiol. Pharmac.*, **26** : 96, 1982.