increased with ATX II concentrations above $5 \times 10^{-8}$ M at 1.8 and 3.6 mM $[Ca^{++}]_e$. The reason for this lack of $[Ca^{++}]_e$ influence on the positive inotropic effect could simply be that the direct enhancement of force of contraction by elevated $[Ca^{++}]_e$ may just balance the smaller effect of ATX II at high $[Ca^{++}]_e$. The incidence of contracture does not necessarily prove a direct increase of toxicity of ATX II at high $[Ca^{++}]_e$[8] but may indicate that the calcium sequestering processes of the muscle are closer to decompensation.

The influence of $Ca^{++}$ on the ATX II-induced prolongation in action potential duration was also tested after the full effect had already been established (fig. 2). The stimulation frequency was lowered from 1 to 0.1 Hz because the ATX II effect on the action potential duration is more prominent at low frequencies[11], although contractile augmentation is also obscured by the direct effect of stimulation frequency on force of contraction[12]. When $[Ca^{++}]_e$ was elevated from 1.8 to 3.6 mM, the lengthened action potential shortened rapidly. The contraction amplitude showed a small transient increase which probably reflected the direct effect of raised $[Ca^{++}]_e$, but this was soon overcome by the diminished efficacy of ATX II. The reverse effects were observed when lowering $[Ca^{++}]_e$ back to 1.8 mM. Similar results were obtained in 4 additional experiments.

The $Ca^{++}$-dependence of the ATX II effect demonstrated here for heart muscle has also been observed in myelinated nerve fibers[13]. The mechanism of interaction between $Ca^{++}$ and ATX II is not clear. Calcium ions have been shown to delay inactivation of the sodium current in nerve membranes, and this may involve negative surface charges associated with the inactivating gate[14]. It could be speculated that the strongly basic polypeptide ATX II requires the same negative surface charges in order to become effective in delaying sodium current inactivation and thus prolong the action potential duration.


1 Ravens, U., Naunyn-Schmiedebergs Arch. Pharmak. *296* (1976) 73.
2 Shimizu, T., Iwamura, N., Toyama, J., Yamada, K., and Shibata, S., Eur. J. Pharmac. *56* (1979) 7.
3 Fujiwara, M., Muramatsu, I., Hidaka, H., Ikushima, S., and Ashida, K., J. Pharmac. exp. Ther. *210* (1979) 153.
4 Bergman, C., Dubois, J.M., Rojas, E., and Rathmayer, W., Biochim. biophys. Acta *455* (1976) 173.
5 Romey, G., Abita, J.P., Schweitz, H., Wunderer, G., and Lazdunski, M., Proc. natl Acad. Sci. USA *73* (1976) 4055.
6 Hashimoto, K., Ochi, R., Hashimoto, K., Inui, J., and Miura, Y., J. Pharmac. exp. Ther. *215* (1980) 479.
7 Honerjäger, P., Rev. Physiol. Biochem. Pharmac. *92* (1982) 1.
8 Alsen, C., Beress, L., Fischer, K., Proppe, D., Reinberg, T., and Sattler, R.W., Naunyn-Schmiedebergs Arch. Pharmak. *295* (1976) 55.
9 Scriabine, A., van Arman, C.G., Morgan, G., Morris, A.A., Bennett, C.D., and Bohidar, N.R., J. cardiovasc. Pharmac. *1* (1979) 571.
10 Reiter, M., and Stickel, F.J., Naunyn-Schmiedebergs Arch. Pharmak. *260* (1968) 342.
11 Beress, L., Ritter, R., and Ravens, U., Eur. J. Pharmac. *79* (1982) 265.
12 Koch-Weser, J., and Blinks, J.R., Pharmac. Rev. *15* (1963) 601.
13 Ulbricht, W., Schmidtmayer, J., J. Physiol., Paris *77* (1981) 1103.
14 Shoukimas, J.J., J. Membrane Biol. *38* (1978) 271.


0014-4754/83/080893-02$1.50+0.20/0


## Is prostacyclin subserving a vasodilator effect of methoxamine involving alpha adrenoreceptors?[1]

E. Borda, L. Sterin-Borda, M.F. Gimeno, M. Lazzari and A.L. Gimeno[2]

*Centro de Estudios Farmacológicos y de Principios Naturales (CEFAPRIN), Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (CONICET) and Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina. Serrano 665/69, (1414) Buenos Aires (Argentina), November 2, 1982*

*Summary.* The influence of methoxamine on the contractile tension of isolated rat abdominal aorta, and on its capacity to produce a platelet antiaggregating substance, were explored. Methoxamine stimulated platelet antiaggregation and diminished arterial tone. The last action was blocked by phentolamine as well as by inhibitiors of cyclo-oxygenase and prostacyclinsynthetase.

Catecholamines are known to stimulate prostaglandin (PG) biosynthesis and release in a variety of animal species and tissues[3]. However, the mechanism by which catecholamines stimulate the biosynthesis of PGs is unclear. They may participate as a phenolic cofactor required for the cyclo-oxygenation of arachidonic acid[4] or alternatively could act by influencing the mechanical activity of the tissue via the stimulation of adrenoreceptors[5]. Although it is difficult to differentiate between these two possibilities, we decided to explore whether an atypical mechanical response of rat aorta to methoxamine is evoked by the activation of alpha adrenoreceptors accompanied by the stimulation of the synthesis and/or release of platelet antiaggregating material.

*Methods.* Abdominal aortae were obtained from male albino rats of the Wistar strain weighing between 200 and 250 g. The animals were stunned by a blow on the neck and their vessels were removed, dissected en bloc and immersed in Krebs-Ringer-Bicarbonate solution (KRB) composed as reported elsewhere[6]. Arterial strips were mounted vertically in a muscle bath containing 20 ml of KRB solution gassed with 5% of $CO_2$ in oxygen and kept at 37 °C and pH 7.4. The preparations, clamped between a stationary holder and an isometric force transducer, were 2 cm long. The contractile activity was recorded as previously reported[7]. After an initial preload of 1 g, the arterial strips underwent a stress relaxation and the tension stabilized after 90 min around 600 mg. The tonic changes of the vessels elicited by experimental additions were expressed in mg of tension above or under the basal resting tension or initial preload (0 mg in the figs). Cumulative dose-response curves for methoxamine (Burroughs Wellcome Co.) were constructed

for untreated control preparations as well as for those previously exposed to drugs known to block prostacyclin ($PGI_2$) synthesis, cyclooxygenase activity or alpha and beta adrenoreceptors. Indomethacin at $10^{-6}$ M (Merck Sharp and Dohme), acetylsalicylic acid (ASA) at $1.8\times10^{-4}$ M (Sigma Chemical Co.), propranolol at $10^{-7}$ M (Sigma Chemical Co.), phentolamine at $10^{-6}$ M (Sigma Chemical Co.), and tranylcypromine at $2.5\times10^{-4}$ M (Smith Kline French), were used. These agents were added to the tissue bath 30 min before methoxamine.

To determine platelet antiaggregation, strips of abdominal aorta (10–12 mg dry wt), with or without methoxamine ($10^{-6}$ M), were incubated for 20 min in 200 µl of KRB solution at room temperature and at pH $8.0\pm0.1$. The supernatant from each reaction mixture was removed and 10 µl of the incubate were placed in the cuvette of a Chronolog aggregometer containing 450 µl of platelet-rich-plasma from normal donors adjusted to 300,000/mm$^3$ (counted in a Coulter Counter C) with autologous platelet-poor-plasma. The addition was made 45 sec before delivering adenosine-diphosphate (ADP, Sigma Chemical Co.) at a final concentration of 5 µM as the aggregating agent. Maximal light transmission through the sample was recorded as a measurement of platelet aggregation. The maximal control aggregating action of ADP was considered as 100% and the antiaggregating capacity of aortic strips exposed or not to methoxamine was determined and expressed as a percent of the change. Interassay coefficient of variation of the method was less than 10%. When an antiaggregatory activity was detected in the samples they were retested after an incubation of 60 min at 37 °C. The PRP stability after 1 h at 37 °C was adequate because aggregation values were comparable to those of controls. All measurements were made in the same platelet-rich-plasma. More details have been reported elsewhere[8]. Results were compared employing Student's t-test. Mean experimental values were considered significantly different when $p=0.05$ or less.

*Results.* Figures 1 and 2 show, in a dose related manner, the contractile influence of methoxamine on strips of abdominal aortae from male rats. Methoxamine ($10^{-8}$ M–$10^{-4}$ M) produced a dose-dependent relaxation of the vessels. Figure 1 also depicts the significant inhibition of the vasodilator effect of methoxamine in preparations exposed to phentolamine ($10^{-6}$ M), an alpha adrenoreceptor antagonist. On the contrary, the beta adrenoreceptor blockade with propranolol ($10^{-7}$ M) failed to modify the vascular relaxation induced by the adrenergic agonist. The incubation during 30 min with indomethacin ($10^{-6}$ M), ASA ($1.8\times10^{-4}$ M) or tranylcypromine ($2.5\times10^{-4}$ M), also inhibited significantly the vasodilator effect evoked by methoxamine (fig. 2).

The table shows comparatively the production of a platelet antiaggregating material by the isolated rat abdominal aorta in the presence and absence of methoxamine. It can be seen that aortic strips alone were able to prevent the aggregatory influence of ADP. Furthermore, when methoxamine ($10^{-6}$ M) was added to the medium containing aortic strips the antiaggregatory capacity of the vessels was significantly increased. Methoxamine alone failed to modify platelet aggregation.

*Discussion.* It is generally accepted that in arterial smooth muscle, alpha adrenoreceptor stimulation induces contraction, while the adrenoreceptor activation of beta ones evokes relaxation.

The present report documents that methoxamine relaxes the isolated abdominal aorta of the rat. The fact that the vasodilating action of methoxamine was inhibited by an alpha adrenoreceptor blocker such as phentolamine, but not by a beta adrenoreceptor antagonist like propranolol, suggests that the relaxing influence involves the participation of alpha rather than that of beta adrenoreceptors.

Platelet-antiaggregation evoked by the supernatant of rat abdominal aorta in the presence and absence of methoxamine

| Samples | % of aggregation* | n |
|---|---|---|
| Control | 100 | 4 |
| Aortic strips alone | $31.3\pm1.5$ | 4 |
| Aortic strips plus methoxamine | $3.3\pm0.2$ | 4 |

*Values expressed as percent of changes between the platelet antiaggregatory capacity of the aortic strips with and without methoxamine, against the maximal control ADP-induced aggregation considered as 100%. The test was also performed after 60 min of incubation at 37 °C and showed the absence of antiaggregatory capacity in the experimental samples. n, Number of experiments. For other details see Methods.

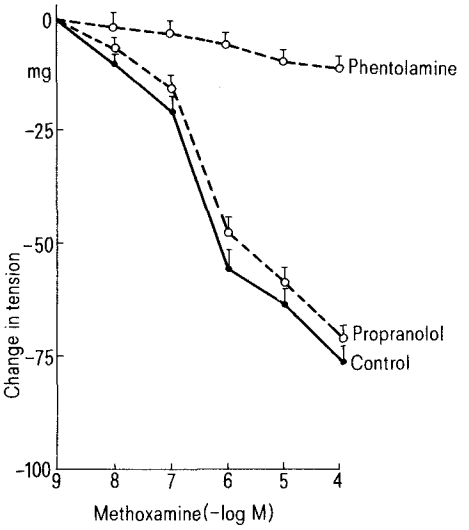


Figure 1. Cumulative dose-response curves of methoxamine on the isometric tension developed by isolated rat abdominal aorta. Influences of phentolamine and propranolol. Points and bars represents means ± SEM respectively; N, 6 in each group. For other details see Methods.

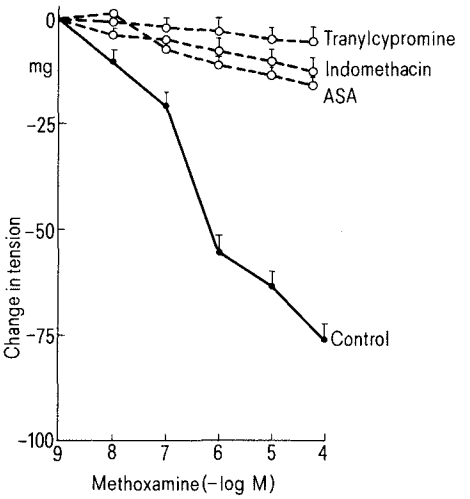


Figure 2. Cumulative dose-response curves of methoxamine on the isometric tension developed of isolated rat abdominal aorta. Influences of indomethacin, acetylsalicylic acid (ASA) and tranylcypromine. For further details see legend of figure 1.

Atypical contractile responses to beta adrenoreceptor agonists were described by us in coronary arteries[9], uterus[10] and vas deferens[11]. However, no currently available studies have described a similar situation regarding alpha adrenoreceptor agonists.

The fact that inhibitors of cyclo-oxygenase, namely indomethacin and ASA[12], abolished the vasodilating effect of methoxamine in abdominal aorta, suggests the possibility that this agonist produces its action by influencing initial reactions involved in the biosynthesis of vasodilator prostaglandins. Prostacyclin ($PGI_2$), synthesized by vascular endothelial cells, is a product of arachidonic acid metabolism with potent dilating effects on blood vessels of a variety of mammalian species[7,13,14]. The fact that tranylcypromine, a nonspecific inhibitor of $PGI_2$ synthetase[15], abolished the vasodilation induced by methoxamine, suggests that $PGI_2$ may participate in this influence.

The additional finding demonstrating that abdominal aortic strips to generate an unstable product with antiaggregatory capacity which is increased in the presence of methoxamine, supports the notion advanced.

The finding that all the inhibitors of PG synthesis tested, as well as the alpha adrenoreceptor blockade, abolished the vasodilating effect of methoxamine, suggests that the mechanism by which this agonist stimulates the output of $PGI_2$, could be mediated by an activation of alpha adrenoreceptors similar to that observed in the rabbit spleen[16]. It is therefore plausible that methoxamine decreases arterial tone, interacting with alpha adrenoreceptors and producing subsequently the release of prostacyclin.

1 This work has been supported by grant 6638 from CONICET (Argentina).
2 To whom reprints requests should be addressed.
3 Westfall, T.C., Physiol. Rev. *57* (1977) 659.
4 Baumann, J., Bruchhausen, F., and Wurm, G., Naunyn Schmiedeberg's Arch. Pharmak. *307* (1979) 73.
5 Flower, R.J., and Blackwell, G.I., Biochem. Pharmac. *25* (1976) 285.
6 Borda, E.S., del Agostini, M.C., Gimeno, M.F., and Gimeno, A.L., Pharmac. Res. Commun. *13* (1981) 981.
7 Borda, E.S., del Agostini, M.C., Sterin-Speziale, N., Gimeno, M.F., and Gimeno, A.L., Prostaglandins *18* (1979) 829.
8 Gimeno, M.F., Sterin-Borda, L., Borda, E.S., Lazzari, M.A., and Gimeno, A.L., Prostaglandins *19* (1980) 907.
9 Borda, L., Schuchleih, R., and Henry, P., Circulation Res. *46* (1980) 878.
10 Borda, E.S., Sauvage, J., Sterin-Borda, L., Gimeno, M.F., and Gimeno, A.L., Eur. J. Pharmac. *56* (1979) 61.
11 Borda, E.S., del Agostini, M.C., Gimeno, M.F., and Gimeno, A.L., Pharmac. Res. Commun. *13* (1981) 487.
12 Vane, J.R., Nature New Biol. *231* (1971) 232.
13 Moncada, S., Br. J. Pharmac. *76* (1982) 3.
14 Sterin-Borda, L., Borda, E.S., Gimeno, M.F., Lazzari, M.L., del Castillo, E., and Gimeno, A.L., Diabetologia *22* (1982) 56.
15 Gryglewsky, R.J., Bunting, S., Flower, R., and Vane, J.R., Prostaglandins *12* (1976) 685.
16 Brückner-Schmidt, R., Jackisch, R., and Hertting, G., Naunyn Schmiedeberg's Arch. Pharmak. *316* (1981) 1.

0014-4754/83/080894-03$1.50 + 0.20/0


## The effect of propylthiouracil on glutathione S-transferase activity of rat spleen in vitro and in vivo

K. Kariya, E. Lee and T. Matsue

*Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kobe-Gakuin University, Ikawadani-cho, Nishi-ku, Kobe 673 (Japan), December 28, 1982*

*Summary.* Propylthiouracil (PTU) inhibited glutathione (GSH) S-transferase (EC 2.5.1.18) activity of rat spleens in a concentration dependent manner in vitro. PTU (1.5 mmoles/kg) treatment of rats for 1 or 2 weeks caused a decrease in leukocyte number and spleen weight. Nevertheless, GSH S-transferase activity was not affected by the same treatment.

GSH S-transferases are present in various organs of many species[1,2], and the purification[3,4] and characteristics[5] of these enzymes have been well established. These enzymes catalyze the conjugation of a wide variety of electrophilic foreign compounds with GSH[1,6,7]. It is established that the role of these enzymes is to protect physiological nucleophiles by conjugating foreign compounds, including pesticides and carcinogens. Recently, Yamada and Kaplowitz have reported that PTU inhibited GSH S-transferase activity by competing with GSH as a substrate in reactions catalyzed by the enzymes[8].

On the other hand, it is recognized that thiono-sulfur-containing antithyroid drugs such as PTU and methimazole cause low level agranulocytosis and granulocytopenia during the treatment of hyperthyroidism[9,10]. The postulated mechanism of PTU action is that it inhibits the synthesis of thyroid hormones by preventing thyroid peroxidase activity[11-13]. However, the mechanism by which PTU induces these adverse reactions is not yet known.

In relation to studies of the mechanism producing these effects by PTU, we found a decrease in spleen weight of rats treated with PTU for 1 or 2 weeks, but a recovery of that weight 2 weeks after the treatment. This paper describes the relationship between PTU and GSH S-transferase activity in rat spleens, with regard to hematopoiesis.

*Materials and methods.* PTU and GSH were purchased from Sigma Chemical Co. 1-Chloro-2,4-dinitrobenzene (CDNB) was from Aldrich Chemical Co. All other reagents were of analytical grade, and available in our laboratory. Isolated spleens of rats were homogenized with 9 vols of 0.25 M sucrose using a Potter-Elvehjem homogenizer with a Teflon pestle at 4 °C. A preliminary experiment revealed significant activity of GSH S-transferase in the various subcellular fractions, especially the supernatant produced at 105,000 × g for 60 min, referred to as the soluble fraction, where more than 60% of the total enzyme activity occurred (data not shown). Therefore, in subsequent experiments, the soluble fraction was used as an enzyme source for GSH S-transferase activity. The standard assay medium for measurement of GSH S-transferase consists of 100 mM potassium phosphate buffer (pH 6.5), 1 mM GSH, 1 mM CDNB as the substrate, and the enzyme source (about 100 µg