EFFECTS OF AGING ON ATP SENSITIVE K⁺ CHANNELS AND ON PROSTANOID PRODUCTION IN THE RAT MESENTERIC BED.

Ethel C. Feleder^{1,2}, Horacio A. Peredo¹, Victoria E. Mendizábal¹ and Edda Adler-Graschinsky¹

¹ Instituto de Investigaciones Farmacológicas

Junín 956 5° piso

1113 Buenos Aires, Argentina

² Cátedra de Farmacología

Facultad de Farmacia y Bioquímica

Universidad de Buenos Aires, Argentina

ABSTRACT

The aim of the present work was to evaluate, in the rat isolated mesenteric bed, whether increasing age is associated with alterations in the ATP sensitive K⁺ channels functionality. Moreover, studies were performed in order to evaluate the effects of aging on the synthesis of vascular prostanoids as well as on its possible contribution to the pressor responses of this vascular bed. Male Wistar rats of 3 month (adults) and 24 month (aged) were used. Although no differences were found among adult and aged rats in pressor responses to 2-30 nmol noradrenaline and to 40-160 nmol KCl, the relaxant responses to the K⁺ channel opener, 10⁻⁶ M cromakalim, were significantly diminished in the aged group compared to the adults. On the other hand, whereas PGF₂ α and 6-keto PGF₁ α production was not modified with age, the thromboxane B₂ and prostaglandin E₂ production in the mesenteric bed from 24 month old rats was significantly increased compared to the adult group. Furthermore, the cyclooxygenase synthesis inhibitor, 10⁻⁵ M indomethacin reduced the pressor responses induced by noradrenaline in the mesenteric beds from adults but not from aged rats. It is concluded that there is an age related reduction in the functionality of the ATP sensitive K⁺ channels in the rat mesenteric bed. In addition, aging produces an increase in the release of vasoconstrictor as well as of vasodilator prostanoids, whose contribution to noradrenaline induced pressor responses appears to be less relevant in the older animals.

INTRODUCTION

Increasing age is associated with functional changes in vascular reactivity (1, 2). For instance, patch-clamp experiments have shown that the hyperpolarization caused by ATP sensitive K⁺ channel openers, such as cromakalim, is decreased in mesenteric arteries isolated from aged rats (3). A reduction in the number of functional ATP sensitive K⁺ channels has also been reported for patches of skeletal muscle fibers isolated from old rats (4).

Regarding the influence of age on prostanoid production, in spite of controversial results, the available information is that aging modifies the output of vasoconstrictor and vasodilator prostaglandins from rat vascular tissues. For instance, the output of the vasoconstrictor prostaglandin F₂ α is increased with age in the rat vasculature (5).

The aim of the present work was to evaluate whether in the rat isolated mesenteric bed, in addition to the electrophysiological alterations found in vascular tissues from old rats (3,4), the functionality of the ATP sensitive K⁺ channels is also decreased with age. Moreover, studies were performed in order to evaluate the effects of aging on the synthesis of vascular prostanoids as well as on its possible contribution to the pressor responses of the mesenteric bed. Pressor responses to noradrenaline and to KCl were used as a parameter in order to evaluate the effects of potassium channel openers and of prostanoid synthesis inhibitors.

MATERIALS AND METHODS

Mesenteric Vascular Bed Preparation

Male Wistar rats of 3 months (adults) and 24 months (aged) were used. The animals were anesthetized with ether and the mesenteric vascular bed was isolated as described by McGregor (6). The abdominal cavity was opened and a polyethylene cannula was inserted into the superior mesenteric artery before the removal of the whole mesenteric bed, which was cut close to the intestinal border of the mesentery.

The isolated preparation was perfused at a rate of 2 ml/min, using a peristaltic pump (Manostat) with Krebs-

To whom all correspondence should be addressed:

Ethel C. Feleder, MD

Instituto de Investigaciones Farmacológicas

Junín 956, 5° piso

1113, Buenos Aires

Argentina

Telephone number: 054-1-961-5949

054-1-961-6784

Fax number: 054-1-963-8593

Henseleit solution whose composition was (10^{-3} M): NaCl 118; KCl 4.7; MgSO_4 1.2; NaHCO_3 25; NaH_2PO_4 1.0; CaCl_2 2.6; glucose 11.1; EDTA disodium salt 0.004 and ascorbic acid 0.11. The solution was gassed with a 95% O_2 :5% CO_2 mixture at 37°C . The final pH was 7.4.

Experimental Protocols

After the equilibration of the mesenteric bed for 60 min, three concentration-response curves in response to a bolus injection of noradrenaline (1, 2, 3, 10, 20 and 30 nmol) were performed, 30 min apart. First curves were carried out in the presence of saline and considered as the controls. Second curves were performed in the presence of the K^+ channel opener cromakalim (10^{-6} M) and third curves were obtained under the simultaneous addition of cromakalim (10^{-6} M) and the K^+ channel blocker glibenclamide (10^{-7} M). At the end of the experimental protocol, two bolus injections of $6 \cdot 10^{-6}$ mol KCl were administered in order to evaluate the pressor status of the preparation.

In other series of experiments, second concentration-response curves to noradrenaline were performed in the presence of the prostanoid synthesis inhibitor, indomethacin (10^{-5} M).

Differences in perfusion pressure were measured as a parameter of vascular resistance and monitored by means of a Statham pressure transducer (P23AC) connected to a Grass polygraph (Model 7PCPA). The perfusion pressure, approximately 50 mmHg, remained constant during the equilibration period and it was taken as the basal value to assess the changes in perfusion pressure induced by the different agents.

Prostanoid Release Measurement

The isolated mesenteric bed was allowed to equilibrate for 30 min. The basal release of prostanoids was measured in the perfusate collected for a 15 min period immediately after the equilibration.

Samples were acidified to pH 3.5 with 1 M formic acid and extracted 3 times with 2 volumes of chloroform. The chloroform fractions were pooled and evaporated to dryness (7). Reversed-phase HPLC was carried out on a C 18 column (Ultropack Lichrosorb). The solvent system was 1.7 mM H_3PO_4 67.2: acetonitrile 32.8 V/V. The flow rate was $1 \text{ ml} \cdot \text{min}^{-1}$ and UV absorption was measured at 218 nm. Dried samples were resuspended in 0.15 ml mobile phase and injected into the HPLC system.

Authentic standards of prostanoids: 6-keto prostaglandin $\text{F}_{1\alpha}$ (6-keto $\text{PGF}_{1\alpha}$); PGE_2 ; $\text{PGF}_{2\alpha}$ (and thromboxane (TX) B_2) were run along with the samples and a bracket assay was performed to determine the amount of prostanoids in the samples. All values were corrected for recovery loss as determined by parallel standards. Results were expressed as ng of prostanoid per mg of tissue weight and per min of perfusion.

Statistical Analysis

Results are expressed as means $\pm$ S.E.M. Differences between groups were evaluated by two way analysis of variance. A P value smaller than 0.05 was regarded as significant. The EC_{50} value (the concentration of agonist required to cause 50% of the maximum response) was determined from individual concentration-response curves by nonlinear regression analysis. Statistical significance was evaluated by Student's *t*-test for unpaired values.

RESULTS

Pressor Responses of the Mesenteric Bed to Noradrenaline and KCl

As shown in Fig. 1, the pressor responses induced by noradrenaline were concentration dependent and of the same extent in the mesenteric beds isolated from 3 month old rats (adults) as well as from 24 month old rats (aged). Table 1 indicates that no differences with age were observed for either sensitivity (EC_{50}) or maximal pressor responses to noradrenaline.

Moreover, as indicated in Fig. 2, the pressor responses elicited by 40 to 160 nmol KCl were concentration-dependent and had a similar extent in the 3 month (Fig. 2A) compared to the 24 month old rats (Fig. 2B). In order to preclude that the pressor responses to KCl were in part due to noradrenaline released from nerve endings as a consequence of the K^+ induced depolarization, concentration-response curves to KCl were also performed in the presence of the alpha adrenergic antagonist, prazosin. As shown in Fig. 2, neither 10^{-7} M nor 10^{-6} M prazosin modified the pressor responses to KCl in the 3 month (Fig. 2A) as well as in the 24 month old rats (Fig. 2B).

Effects of Cromakalim on the Pressor Responses to Noradrenaline

The ability of the K^+ channel opener cromakalim to reduce the pressor activity elicited by different concentrations of noradrenaline is depicted in Fig. 3. Cromakalim (10^{-6} M) reduced the pressor responses to noradrenaline in mesenteric beds from adults as well as from aged rats. Nevertheless, this effect was significantly diminished in the older animals, in spite of the fact that the pressor responses to noradrenaline had similar values in both groups, as indicated in Table 2. In addition, and as shown in Fig. 4, the ATP sensitive K^+ channel blocker, 10^{-7} M glibenclamide, reduced to 10-20% of control values the relaxation produced by cromakalim on the pressure increases elicited by noradrenaline in adults as well as in aged rats.

Effects of Indomethacin on the Pressor Responses to Noradrenaline and KCl

As shown in Fig. 5A, the prostanoid synthesis inhibitor, indomethacin, at a concentration of 10^{-5} M, reduced significantly the pressor responses to all the concentrations of noradrenaline tested in mesenteric beds from 3

Fig. 1. Concentration response curves for the pressor responses of the mesenteric bed to different concentrations of noradrenaline. The tissues were obtained from 3 month old rats (filled circles, $n=37$) and from 24 month old rats (open circles, $n=12$). Results are means $\pm$ S.E.M. and are expressed as the increase in perfusion pressure (mmHg) measured at a constant flow of 2 ml/min.

Fig. 3. Effects of 10^{-6} M cromakalim on the pressor responses to different concentrations of noradrenaline. The mesenteric beds were obtained from 3 month old rats (filled bars, $n=7$) and from 24 month old rats (open bars, $n=7$). Ordinate: percent of relaxation caused by cromakalim on the pressor responses elicited by noradrenaline. Results are means $\pm$ S.E.M. *: $P<0.05$ compared to the 3 month old rats. The absolute values (mmHg) for the contractions to the lowest (2nmol) and the highest (30 nmol) noradrenaline concentrations are shown in Table 2.

Fig. 2. Effects of 10^{-7} M prazosin (open triangles) and of 10^{-6} M prazosin (open squares) on the pressor responses to different concentrations of KCl. Control values are depicted in filled circles. The mesenteric beds were obtained from 3 month old rats (panel A, $n=8$) and from 24 month old rats (panel B, $n=4$). Results are mean $\pm$ S.E.M.

Fig. 4. Effects of 10^{-7} M glibenclamide on the reductions of the pressor responses to different concentrations of noradrenaline induced by 10^{-6} M cromakalim (open bars, $n=7$). Controls (10^{-6} M cromakalim) are depicted in filled bars ($n=7$). The mesenteric beds were obtained from 3 month old rats (A) and from 24 month old rats (B). Results are means $\pm$ S.E.M. *: $P<0.05$ compared to the corresponding controls without glibenclamide.

Fig. 5. Effects of 10^{-5} M indomethacin on the pressor responses to different concentrations of noradrenaline (open bars). Controls are depicted in filled bars. The mesenteric beds were obtained from 3 month old rats (A, $n=6$) and from 24 month old rats (B, $n=5$). Results are means $\pm$ S.E.M. and are expressed as the increase in perfusion pressure (mm Hg) at a constant flow of 2 ml/min. *: $P < 0.05$ compared to their corresponding controls.

Table 1: EC_{50} and maximal pressor responses induced by noradrenaline in the rat isolated mesenteric bed.

Age	EC_{50} (nmol)	Maximal pressor responses (mmHg)
3 month (n=37)	5.24 ± 1.62	61.53 ± 6.54
24 month (n=12)	4.27 ± 1.25	69.29 ± 9.25

Table 2: Absolute values for the pressor activity produced by 2 and 30 nmol noradrenaline on mesenteric beds obtained from 3 month old rats (adults) and from 24 month old rats (aged).

Noradrenaline administration	Pressor responses (mmHg)	
	Adults (n=7)	Aged (n=7)
2 nmol	25.4 ± 7.1	27.86 ± 5.0
30 nmol	60.3 ± 5.4	69.29 ± 9.3

Fig. 6. Effects of 10^{-5} M indomethacin (open bars) on the pressor responses to a single bolus injection of $6 \cdot 10^{-6}$ mol KCl. Controls are depicted in filled bars. The mesenteric beds were obtained from 3 month old rats ($n=6$) and from 24 month old rats ($n=5$). Results are means $\pm$ S.E.M. and are expressed as the increase in perfusion pressure (mmHg) at a constant flow of 2 ml/min.

Fig. 7. Basal release of prostanoids from mesenteric beds obtained from 3 month (filled bars, $n=8$) and from 24 month old rats (open bars, $n=7$). Results are means $\pm$ S.E.M. and are expressed as ng of prostanoid .mg of tissue⁻¹. min of perfusion⁻¹. * $P < 0.01$ compared to the 3 month old group.

month old rats. On the contrary, and as depicted in Fig.5B, in mesenteric beds from 24 month old rats indomethacin did not modify the pressor responses elicited by noradrenaline. On the other hand, Fig. 6 shows that the pressor activity elicited by $6 \cdot 10^{-6}$ mol KCl was not modified by indomethacin either in adult or in aged rats.

Prostanoid production

Fig. 7 shows the basal prostanoid production in mesenteric beds from 3 month and 24 month old rats. The

prostanoids released to the perfusion medium were 6-keto PGF_{1α} (stable metabolite of PGI₂ or prostacyclin), TXB₂ (stable metabolite of TXA₂), PGF_{2α} and PGE₂. In the 24 month old animals, the release of TXB₂ and of PGE₂ was significantly increased ($p < 0.01$) as compared to the 3 month old group. No changes were found in either 6-keto PGF_{1α} or PGF_{2α} production.

DISCUSSION

The present results obtained in mesenteric beds from 24 month old rats show that aging reduces the relaxation elicited by the K⁺ channel opener cromakalim on noradrenaline induced contractions. In addition, aging was found to be linked to an increase in the production of the vasoconstrictor prostanoid TXA₂ as well as of the vasodilator PGE₂, although the contribution of prostanoids to the pressor responses induced by noradrenaline was evident in the adult but not in the old rats.

The fact that the relaxation induced by 10⁻⁶ M cromakalim on the pressor responses to noradrenaline was diminished in the old rats could be due to a reduction in the functionality of the ATP sensitive K⁺ channels with age. In support of this view, electrophysiological evidence shows that the hyperpolarization caused by 10⁻⁶ M cromakalim on contractions elicited by 10⁻⁵ M noradrenaline was diminished in mesenteric arteries isolated from aged rats (3). Moreover, a reduction in the functionality as well as in the number of functional ATP sensitive K⁺ channels was reported for skeletal muscle fibers of aged rats (4). In addition, the fact that the K⁺ channel blocker 10⁻⁷ M glibenclamide (8) reduced the relaxations produced by cromakalim in adult as well as in aged rats indicates that the effect of cromakalim is due in both cases to the opening of the ATP sensitive K⁺ channels. The observation that in mesenteric beds from adult rats the prostanoid synthesis inhibitor, 10⁻⁵ M indomethacin reduced the pressor responses to noradrenaline but not to KCl agrees with the report that pressor activity elicited by both agents obey to different mechanisms (9). Inhibition by indomethacin of contractile responses to noradrenaline has also been reported for the rat splenic vasculature (10) and rat isolated mesenteric arteries (11). Moreover, the fact that the reduction caused by indomethacin on the pressor responses to noradrenaline was manifested in adult but not in aged rats could suggest that the contribution of prostanoids on noradrenaline pressor activity is less relevant in old rats. On the other hand, the observation that the mesenteric beds isolated from 24 month old rats produced more TXB₂ and PGE₂ than the tissues from 3 month old rats is in agreement with the progressive increase with age in the production of prostanoids that has also been reported for TXB₂ in the effluent of perfused rat mesenteric beds (9) and for PGE₂ in mouse splenocytes (12). In addition, cultured endothelial cells from human umbilical veins produce more TXB₂ after a procedure of *in vitro* aging (13).

The observation that the contractile responses to noradrenaline as well as to KCl were entirely preserved in aged rats agrees with the lack of alteration with age

reported for the responses to noradrenaline in rat mesenteric artery rings (14) and in the rat aorta (15). Nevertheless they are opposite to those reported by Atkinson et al. (16) who found an age-related increase in the contractile responses to noradrenaline in the isolated mesenteric bed. The discrepancy between ours and their results could rely on the experimental protocol employed in either case. I.e. the perfusion flow used by Atkinson was 4 ml/min whereas ours was 2 ml/min. Further experiments deserve to be done in order to elucidate this point.

In conclusion, these results support, from a pharmacological point of view, an age-related reduction in the functionality of the ATP-sensitive K⁺ channels in the rat mesenteric bed. This alteration is accompanied by increments in the release of vasoconstrictor as well as of vasodilator prostanoids, whose contribution to the pressor responses induced by noradrenaline is not apparent in the older animals.

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