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CHANGES IN CORONARY VASCULAR RESISTANCE ASSOCIATED WITH PROLONGED HYPOXIA IN ISOLATED RAT HEARTS: A POSSIBLE ROLE OF PROSTAGLANDINS

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SUMMARY

Hypoxia caused an initial dilatation in the coronary circulation of perfused male rat hearts but within 15 minutes the coronary vessels became strongly constricted. In hearts from very young (30 days) animals only dilatation was seen. Physiological levels of progesterone in the perfusate prevented the constriction whereas estradiol and testosterone had little effect. Blockade of adrenergic alpha receptors or angiotensin receptors did not prevent the constriction. Two structurally different inhibitors of prostaglandin synthesis, indomethacin and aspirin, and three drugs which can interfere with prostaglandin action, chloroquine, procaine, and propranolol blocked the constriction. Thromboxane A2, a product of PG synthesis, had been reported to be a coronary vasoconstrictor but four drugs which inhibit thromboxane A2 synthesis, dipyridamole, benzydamine, N-0164 and imidazole were not able to prevent the hypoxia-induced constriction. This form of hypoxic coronary constriction seems not to be related to α-adrenergic, angiotensin or thromboxane A2 effects. It may depend on some other product of the prostaglandin pathway.

INTRODUCTION

Oxygen insufficiency is generally considered to result in dilatation of the coronary vasculature. The mechanism is uncertain but increased adenosine formation may be involved (1). However most studies of the effects of oxygen lack use only a short period of exposure to anoxic or hypoxic media. We here report that if, in isolated rat hearts, hypoxic perfusion is prolonged, the initial dilatation passes off and an intense vasoconstriction results. Possible mechanisms of this constriction have been explored and the two most likely, activation of an alpha adrenergic mechanism or production of thromboxane A2, ruled out. The constriction may be related to overproduction of an as yet unidentified prostaglandin. Constriction could be prevented by progesterone but not by estradiol or testosterone.

These observations may have relevance to medicine. There is increasing evidence that angina pectoris and myocardial infarction may often be due to active coronary constriction (2-5). These clinical states are associated with myocardial hypoxia and since experimental hypoxia has been consistently reported to cause dilatation (1), the simultaneous occurrence of hypoxia and constriction has been difficult to explain. Our finding that prolonged hypoxia can cause constriction may throw new light on this question.

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METHODS

Animals

Male Wistar rats (Canadian Breeding Co., St.Constant, Quebec) were used in this study. For most experiments 3 month (average 97 days) old rats were used. In some experiments 1 month (30 days) old rats were also studied.

Heart Perfusion

Hearts were removed from ether-anesthetized animals and the aorta cannulated (Langendorff method). The coronary arteries were perfused by Krebs-Henseleit buffer through which a 95% O_2/5% CO_2 mixture was bubbled. For hypoxic perfusion the oxygenated buffer was replaced with medium bubbled with nitrogen (pO_2 20-30 mm Hg). The pH of the normal medium was 7.4 and that of the hypoxic medium 7.2. The temperature of the entire system was 37°C. The perfusion pressure was recorded via a side arm of the aortic cannula. The flow (about 5 ml/g tissue/min) was adjusted before starting the experiment to give an initial pressure of 80-90 mm Hg and was not changed thereafter. Detailed protocols are given in the results section.

Drugs and chemicals

The following drugs were used: indomethacin, aspirin, imidazole, testosterone proplonate, oestradiol benzoate and progesterone, chloroquine, procaine (Sigma Chemicals, St.Louis, Missouri), propranolol (Inderal (R), Ayerst Laboratories, Montreal), benzydamine (Acraf Pharmaceuticals, Montreal), sodium P-benzyl-4-[3-oxo-2 (4-chloro-benzyl)-3-phenyl propyl] phenyl phosphate (N-0164, Nelson Laboratories, Irvine, California), dipyridamole (Persantine (R), Boehringer Ingelheim, Montreal).

RESULTS

Effects of hypoxia in hearts from young and mature animals

The effect of hypoxia on perfusion pressure in hearts from young and mature rats is shown in figure 1. After an initial 30 minute perfusion with oxygenated buffer, the hearts were perfused with unoxygenated buffer. Hearts from young animals (6 rats) exhibited an immediate and significant ($p < 0.001$ at 5 min., Student's t test) drop in perfusion pressure after switching to unoxygenated buffer. This persisted throughout the perfusion period. Reoxygenation after 60 minutes resulted in an increase in perfusion pressure back to levels not significantly different from control ($p > 0.05$, not shown). Hearts from mature animals (12 rats) exhibited an initial drop in perfusion pressure ($p < 0.001$ at 5 min.) similar to that observed in young animals. However within 15 minutes the perfusion pressure began to rise and reached levels substantially above control. Maximum perfusion pressure (40 mm Hg above control, $p < 0.001$) was reached after 30-40 minutes and was sustained for the remainder of the hypoxic perfusion period. Results with reoxygenation at this point were variable with 7 hearts demonstrating a decline in perfusion pressure and 5 exhibiting no change in pressure after reoxygenation.

The effects in mature hearts were not due to the low pH of the hypoxic buffer. In 2 experiments the normal oxygenated buffer was titrated to pH 7.2 with 1 N hydrochloric acid. This low pH oxygenated buffer caused a slight (less than 10 mm Hg) dilatation which persisted for the full hour of the perfusion.

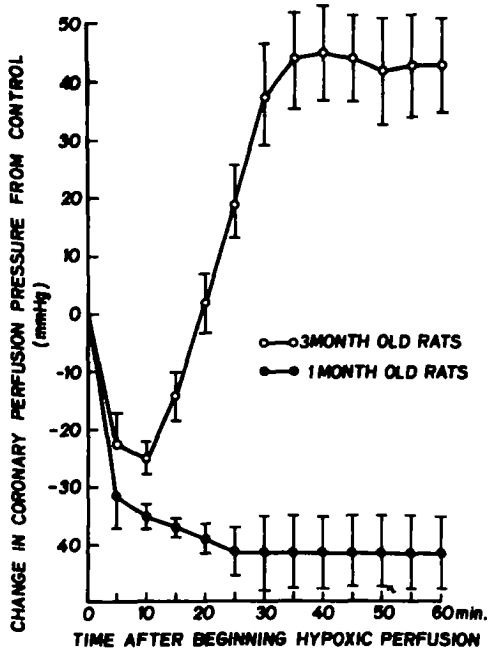

FIG. 1

Effect of age on the coronary artery response to hypoxia in isolated rat hearts. Bars indicate S.E.M. (n = 6 for 30 day old rats and n = 12 for 3 month old rats).

The angiotensin II antagonist saralasin was added to the buffer either 10 minutes before the start of the hypoxic perfusion (2 expts) or 40 minutes after the start of the hypoxic perfusion (2 expts). In neither case was the perfusion pressure different from that observed in experiments without saralasin. The alpha receptor blocking drug phenoxybenzamine was tested in exactly the same way but also had no effect.

Effects of aspirin and indomethacin

Both indomethacin and aspirin, when added to the normal oxygenated buffer, dilated the coronary vessels. Concentrations of aspirin up to 128 µg/ml had only a slight dilating effect (threshold 64 µg/ml, $p > 0.05$) in 4 hearts studied. Indomethacin however was an effective dilator. It was added to the buffer in progressively increasing concentrations, each concentration being present for 10 minutes. Preliminary experiments had shown that the effect of each concentration reached a plateau within 5-10 minutes. At 40 µg/ml the fall in pressure was significant at the $p < 0.001$ level (figure 2). When indomethacin in a concentration of 10 µg/ml was added to the buffer 10 minutes before the start of the hypoxic perfusion, it failed to prevent a rapid dilatation in the early period of hypoxia but did prevent the later phase of constriction (figure 4, 6 experiments). When indomethacin was added to the buffer after the hypoxic constriction had developed, it was able to reverse the constriction (figure 3, 5 experiments). Aspirin at 100 µg/ml also slightly reduced the constriction when added to the buffer before hypoxic perfusion and partially reversed it when added after constriction had developed (4 experiments each).

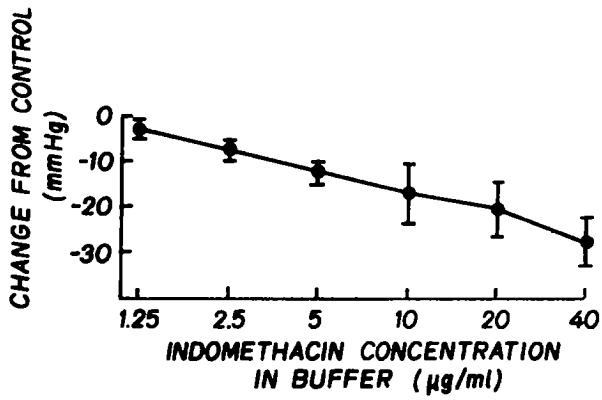

FIG.2

Effect of increasing indomethacin concentrations on the coronary perfusion pressure in isolated rat hearts perfused with normally oxygenated buffer. Each concentration of indomethacin was tested for 10 min. Bars indicate S.E.M. (n,6).

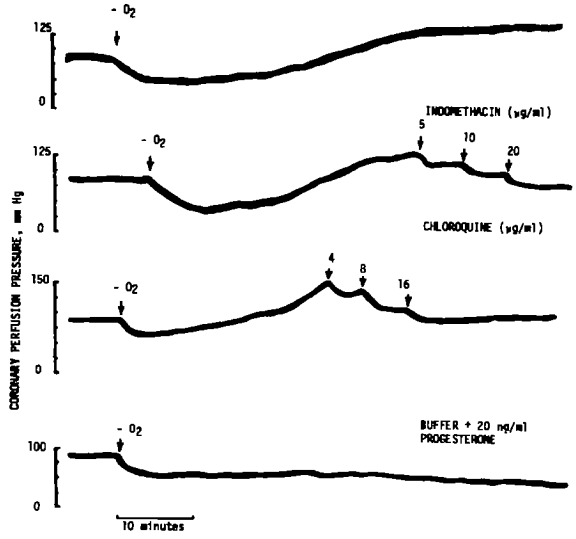

FIG. 3

Coronary perfusion pressure of isolated rat hearts during hypoxia in normal buffer (top) and after the addition of indomethacin and chloroquine. Bottom recording illustrates the effects of hypoxia in the presence of progesterone.

Table 1. Effects of chloroquine, procaine and propranolol on the coronary artery response to hypoxia.

TIME AFTER BEGINNING HYPOXIC PERFUSION (min)	BUFFER ONLY (n = 12)	CHLOROQUINE (10 µg/ml) (n = 4)	PROCAINE (128 µg/ml) (n = 4)	PROPRANOLOL (16 µg/ml) (n = 4)
10	-24.2 ± 3.1	-19.8 ± 7.1	-21.7 ± 6.8	-24.6 ± 7.8
20	1.3 ± 2.6	-24.9 ± 6.3**	-20.5 ± 6.3**	-18.5 ± 8.3**
30	36.1 ± 9.3	-20.7 ± 9.7**	-20.5 ± 11.0**	-12.3 ± 6.1**
40	41.7 ± 9.2	-19.4 ± 5.8**	-14.8 ± 7.3**	-10.0 ± 4.5**
50	39.8 ± 9.7	-20.6 ± 9.1**	-12.7 ± 5.9**	-10.0 ± 4.7**
60	40.6 ± 9.1	-22.4 ± 8.7**	-14.3 ± 7.6**	- 9.7 ± 5.8**

Values in columns represent mean changes in coronary perfusion pressure (in mm Hg) ± SEM.

** indicates highly significant differences ($p < 0.01$) from mean values of drug-free buffer at the specific time interval.

Drugs were present in the buffer before commencing hypoxic perfusion.

Table 2. Effects of thromboxane A2 synthesis inhibitors on the coronary artery response to hypoxia.

TIME AFTER BEGINNING HYPOXIC PERFUSION (min)	BUFFER ONLY (n = 12)	DIPYRIDAMOLE (0.32 µg/ml) (n = 3)	BENZYDAMINE (0.16 µg/ml) (n = 4)	N-0164 (0.16 µg/ml) (n = 3)	IMIDAZOLE (40 µg/ml) (n = 4)
10	-24.2 ± 3.1	-29.8 ± 6.6	-20.8 ± 8.3	-25.3 ± 6.1	-18.7 ± 6.2
20	1.3 ± 2.6	- 4.9 ± 7.1	2.0 ± 1.9	- 6.6 ± 2.0*	- 7.7 ± 3.2*
30	36.1 ± 9.3	29.3 ± 9.0	24.8 ± 9.5*	19.9 ± 7.3**	16.7 ± 6.1**
40	41.7 ± 9.2	42.1 ± 11.7	33.8 ± 8.7*	24.7 ± 6.1**	25.8 ± 7.1**
50	39.8 ± 9.7	46.9 ± 8.4*	38.3 ± 9.0	30.6 ± 5.2*	31.4 ± 7.0
60	40.6 ± 9.1	44.9 ± 9.0	42.7 ± 6.5	36.7 ± 7.1	36.9 ± 10.2

Values in columns represent mean changes in coronary perfusion pressure (in mm Hg) ± SEM

* Indicates significant differences ($p < 0.05$) and ** indicates highly significant differences ($p < 0.01$) from mean values of drug-free buffer at the specific time interval.

Drugs were present in the buffer before commencing hypoxic perfusion.

Effects of membrane-stabilizing drugs that antagonize PG action

We have reported that chloroquine (6), procaine (6) and propranolol (7) can antagonize some PG actions. All three were tested both by adding them to the buffer either 10 minutes before the start of hypoxic perfusion or at the time when maximal hypoxic vasoconstriction had developed. Chloroquine was the most effective agent. At 10 µg/ml it could prevent the constriction when present from the beginning (4 expts, table 1) and at 4 µg/ml it could reverse significantly ($p < 0.05$) an established constriction (4 expts, figure 3). High levels of procaine (128 µg/ml, table 1), and of propranolol (16 µg/ml, table 1) were required for similar effects. All three agents had weak dilating effects on hearts perfused with oxygenated buffer (2 expts each, not shown). None of the drugs altered the early dilatation.

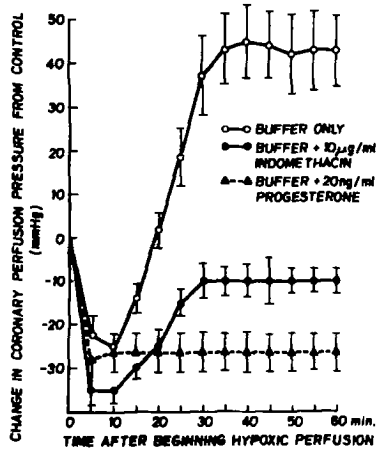

FIG. 4

Effect of indomethacin and progesterone on the coronary artery response to hypoxia in isolated rat hearts. Each drug was present in the buffer before changing to hypoxic perfusion. Bars indicate S.E.M. (n = 12) for control, n = 6 for indomethacin and progesterone experiments).

Effects of thromboxane A2 (TXA2) synthesis inhibitors

Four agents (imidazole, benzydamine, N-0164 and dipyridamole) were used. All four failed to prevent the rise in perfusion pressure when present in the buffer from 10 minutes prior to the start of hypoxic perfusion (Table 2). When added to normally oxygenated buffer in progressively increasing concentrations, dipyridamole, benzydamine and N-0164 all increased the perfusion pressure at low concentrations and decreased it at higher ones (Figure 5, 6 expts with each drug). Imidazole consistently increased the perfusion pressure. When 10 µg/ml indomethacin was present in the buffer all four drugs failed to raise the pressure (Figure 5, 6 expts with each drug).

Effects of gonadal steroids

Testosterone (1 ng/ml to 32 ng/ml, each concentration present for 10 minutes), estradiol (1 ng/ml to 0.32 µg/ml) and progesterone (1 ng/ml to 1.24 µg/ml)

had little effect on pressure in hearts perfused with normal buffer (2 expts each, not shown). Testosterone (16 ng/ml, 5 expts) and estradiol (0.5 ng/ml, 5 expts) did not modify the response to hypoxia when present from 10 minutes before the beginning of hypoxic perfusion. However, progesterone (20 ng/ml, 6 expts, figures 3 and 4) completely prevented the hypoxic constriction and allowed the maximal hypoxic dilatation to persist for the duration of the experiment. Progesterone was less effective in reversing an established constriction and a concentration of 1 µg/ml was required for a clear effect (3 expts, not shown).

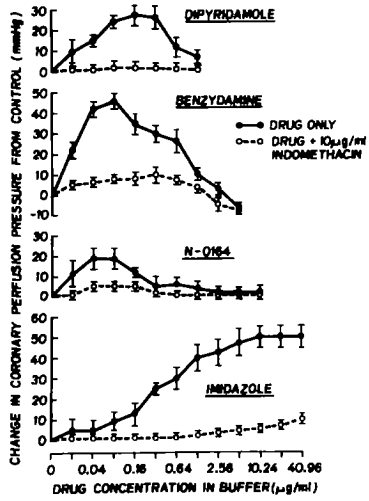

FIG. 5

Effect of TXA2 synthesis inhibitors on the coronary perfusion pressure in isolated rat hearts. Each drug concentration was tested for 10 min. either alone or in the presence of indomethacin. Bars indicate S.E.M. (n = 6 for all experiments).

DISCUSSION

The most important observation reported here is the development of raised coronary resistance in response to prolonged hypoxia. Development of edema is a possible explanation as is vasoconstriction. The rapid effects of indomethacin and chloroquine (fig. 3) and the failure of increased resistance to develop in young or progesterone-perfused hearts argue in favor of constriction as a mechanism. Whether the effect is species specific or is of more general significance can only be tested by further experiment.

The constriction did not appear to be due to the release or formation of either angiotensin II or noradrenaline. Neither angiotensin nor alpha receptor blockade influenced its development. Beta blockade with propranolol was effective only at concentrations at which the drug also has membrane-stabilizing actions.

Thromboxane (TX) A2 causes coronary constriction (8) but four agents which have been reported to inhibit TXA2 formation selectively, imidazole (9), benzydamine (10), N-0164 (10) and dipyridamole (11, 12), all failed to prevent constriction. Indeed they themselves caused constriction when present in

oxygenated buffer at the concentrations at which their thromboxane effect is reported to be selective. It therefore seems unlikely that TXA2 played a role in the hypoxic constriction. Both hypoxia and inhibition of TXA2 synthesis lead to increased production of prostaglandins (PGs) (13); this is partly due to diversion of PG endoperoxides from TXA2 but possibly also partly due to removal of a negative feedback control of the PG synthetic pathway normally exerted by TXA2 (13). PGF2α is a known coronary constrictor agent released during myocardial ischemia (14). Inhibitors of PG synthesis at high concentrations prevented or reversed the constriction. This is consistent with the involvement of a PG or related substance but does not prove it since the drugs have other effects. The high concentrations necessary are not surprising since myocardial PG synthetase is very resistant to aspirin (15). Chloroquine, procaine and propranolol can all behave as PG antagonists (6,7) but they too have many other actions.

Males seem more susceptible than females to coronary artery disease and much attention has been directed either to a possible harmful effect of testosterone or protective effect of estradiol. The failure of estradiol or testosterone to have any effect and the complete prevention of the constriction by physiological levels of progesterone suggest that more attention should be paid to this last steroid.

The findings that hypoxia can cause coronary constriction and that the effect does not occur in young or progesterone-treated hearts suggest that the mechanism may have clinical relevance. Coronary vascular spasm, which tends to occur in mature, male, hypoxic hearts is currently being recognized as an important factor in ischemic heart disease (2-5).

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