REPORT 99/1

EFFECTS OF IQB-9302 ON BASAL AND EVOKED CONTRACTIONS OF ISOLATED RAT AORTA

Number of pages of the report: 11

The present study was performed in compliance with the rules and regulations of Good Laboratory Practices published by OECD (1981) and according to the Real Decreto 822/1993 BOE (May 1993). There were no incidences that could affect reliability of data.

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INTRODUCTION

Local anaesthetics are applied locally and block nerve conduction of sensory impulses from the periphery to the central nervous system. They bind to a specific receptor inside the pore of the Na^+ channel in the nerves, blocking the entry of that ion.

Most of the local anaesthetics produce vasodilation of small arteries as a side effect. This is the reason why they are usually associated to a vasoconstrictor that also decreases the rate of absorption, thus minimizing their systemic toxicity.

IQB-9302 is a new drug derived from bupivacaine currently under pharmacological development. In the present report we describe its effects on isolated rat aorta:

1) Precontracted with 35 mM KCl
2) Precontracted with 1 μM phenylephrine
3) On basal tone.

In all cases IQB-9302 was compared with bupivacaine.

MATERIALS AND METHODS

MATERIALS.-

1.- Jacketed organ baths with a capacity of 40 ml, kept at 37ºC.
2.- Constant temperature water baths with pumps to ensure the optimal heat exchange.
3.- Data adquisition system. MacLab for Macintosh.
4.- Program Chart v3.5.1.
5.- IQB-9302.HCl batch nº 9454.001 from LEBSA. Bupivacaine.HCl, phenylephrine.HCl, carbachol, phentolamine and nifedipine from Sigma, Madrid, Spain.

SOLUTIONS.-

Krebs-bicarbonate solution of the following composition: NaCl 119 mM, KCl 4.7 mM, $MgSO_4$ 1.2 mM, KPO_4H_2 1.2 mM, $CaCl_2$ 1.5 mM, $NaHCO_3$ 24.9 mM, glucose 10.9 mM, was used in all experiments. The solution was continuously bubbled with 95% oxygen and 5% carbon dioxide.

METHODS.-

Male Sprague-Dawley rats weighing 225-300 g from our animal room were used in all the experiments. The animals were killed by asphyxiation in a gas chamber. The thoracic aorta as close as possible to the heart was quickly removed and placed in a Petri dish containing Krebs-bicarbonate solution and the excess fat and connective tissue were removed. Then, the aorta was cut into 3-5 mm-wide rings and two fine stainless steel hooks were carefully introduced into the vessel's lumen. In all experiments the muscles were loaded with 1g and subjected to a 1 h initial equilibration period to allow development of a stable basal tone.

EXPERIMENTAL PROTOCOL.-

Once the tissues were stabilized with an initial incubation period of 60 min, contractions were evoked by adding to the organ bath either KCl so that the final concentration of K^+ was 35 mM (uncorrected for osmolarity) or phenylephrine 1 μM (final concentration). After peak contraction, carbachol 10 μM was added to test the presence of functional endothelium. The preparations were washed twice with fresh Krebs-bicarbonate buffer to allow its relaxation to basal tone and new additions of 35 mM K^+ or 1 μM phenylephrine were repeated to evoke a persistent contraction. Then, the drugs to be tested were added cumulatively (0.1 - 100 μM). Finally, 1 μM nifedipine (in the preparations precontracted with 35 mM K^+) or 1 μM phentolamine (in those precontracted with phenylephrine) were added to test the functionality of the preparations.

In those experiments designed to test the effects of the drugs on basal tone, they were added in the same cumulative way after washing of preparations without further contractions.

ANALYSIS OF DATA.-

Data of contraction in g in the absence (control) or in the presence of drugs were transferred to an Excel worksheet and were transformed into percentage considering 100% the initial contraction, before addition of the drugs. The data of all the experiments were included in the same Excel worksheet and mean $\pm$ SEM was calculated. Statistical analysis of means was performed with the program Statworks for Macintosh using Student's t-test. The level of significance between means was taken at $P \leq 0.05$.

RESULTS

Fig.1 shows original traces of three different experiments Panels A and B show the effects of cumulative concentrations of IQB-9302 on isolated rat aorta precontracted with 35 mM K^+. (A) or bupivacaine on isolated rat aorta precontracted with 1 μM phenylephrine (B) Only the highest concentration tested (10^{-4} M) induced a slight vasorelaxant response in both cases. At the end of the experiment, nifedipine 1 μM (A) or phentolamine 1 μM (B) were added to test the functionality of the preparations; panel (C) shows an experiment in which carbachol 10 μM was added on the peak contraction evoked by 35 mM K^+ to asses the presence of functional endothelium (relaxation induced by NO liberation). After washing out the aorta twice with fresh Krebs-bicarbonate, IQB-9302 was added in a cumulative way. Observe that the compound did not modify the resting tension of the vessel; only at 10^{-4} M a slight increase was produced. Finally, after a new wash out, the preparation was challenged again with 35 mM K^+ to test the functionality of the preparation.

Fig. 2 summarises the results of different experiments comparing the effects of IQB-9302 and bupivacaine on rat aorta precontracted with 35 mM K^+.As can be seen, IQB-9302 exerted a small increase of the K^+-induced contraction (14.8$\pm$6.3%) at the concentration of 10 μM. At 100 μM, both IQB-9302 and bupivacaine induced statistically significant relaxations of 26.6$\pm$6.4% and 22.1$\pm$7.7% respectively, with respect to the initial contraction.

Fig.3 compares the effects of IQB-9302 and bupivacaine on the contraction evoked by 1 μM phenylephrine in rat aorta. A very small increase in the contraction can be noticed for bupivacaine at the concentration of 1 μM (5.4$\pm$2%). Again, both IQB-9302 and bupivacaine, at 100 μM, produced statistically significanta relaxations of 21.9$\pm$4.2% and 21.8$\pm$4.7%, respectively, with respect to the initial contraction.

Fig. 4 shows the effects of IQB-9302 and bupivacaine on the basal tone of rat aorta preparations. As can be seen, only bupivacaine 100 μM induced a significant increase in the basal tone (1.39$\pm$0.6 g).IQB-9302 tended to increase the basal tone at 10^{-4} M, but the difference was not statistically significant.

In all the cases, there were no significant diferences between the effect of IQB-9302 and bupivacaine.

DISCUSSION AND CONCLUSIONS

According to the results presented in this report, it seems that IQB-9302 is acting on the isolated rat aorta in the same way as bupivacaine. Both drugs produced vasodilation only at concentrations as high as 100 μM. These are concentrations that will never be reached sistemically, after local administration of IQB-9302. The only diference observed between both drugs in the present experiments, was the increase in the basal tone induced by bupivacaine at 100 μM. Nevertheless, it is neccesary to wait the results of experiments already programmed, to know the effect on electrically driven left atrium and the blood pressure in anesthetized and conscious rats, before concluding that IQB-9302 is devoid of cardiovascular effects. Also, it could be interesting to perform experiments in smaller arteries (i.e. the third branch of mesenteric arteries), since their contractile regulation differs from larger vessels.

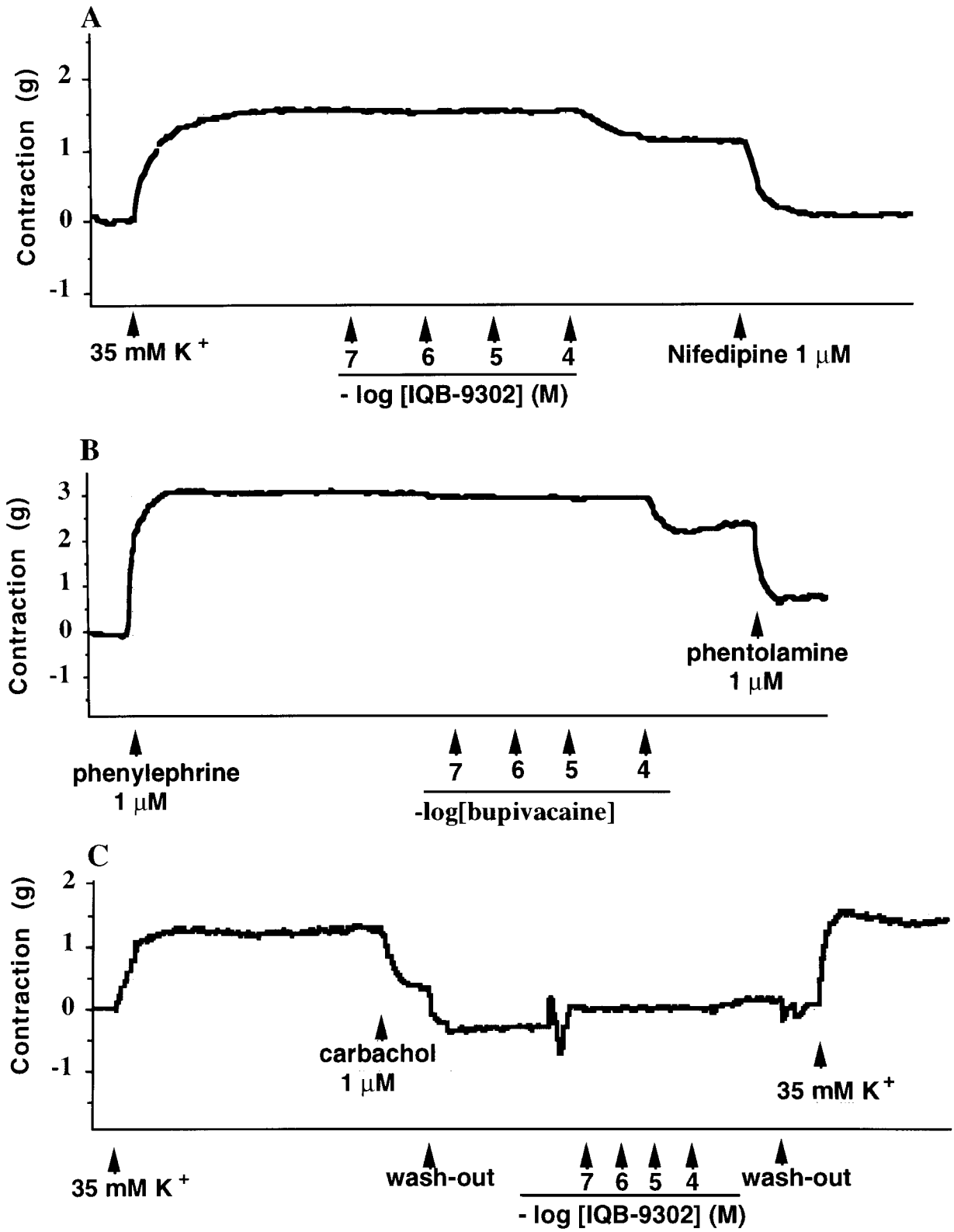

Fig.1.- Original traces of three different experiments on rat aorta precontracted with 35 mM K^+ (A), phenylephrine 1 μM (B) or on basal tone (C).

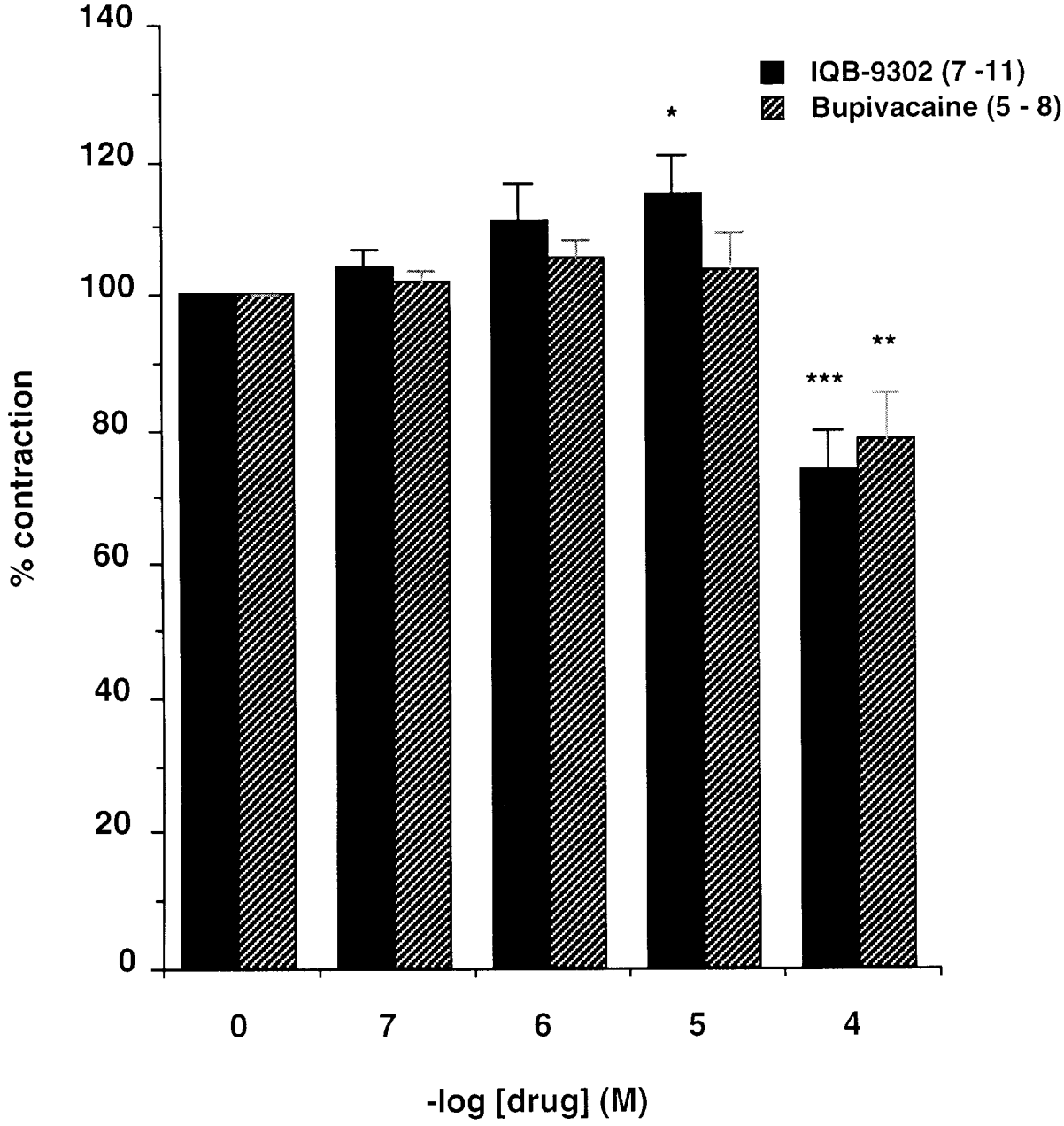

Fig. 2.-Effects of IQB-9302 (■) or bupivacaine (▨) on isolated rat aorta precontracted with 35 mM K^+. The data were calculated from experiments similar to those shown in Fig.1 A; they correspond to means ± SEM of the number of experiments shown in parentheses. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ with respect to their controls, in the absence of drug.

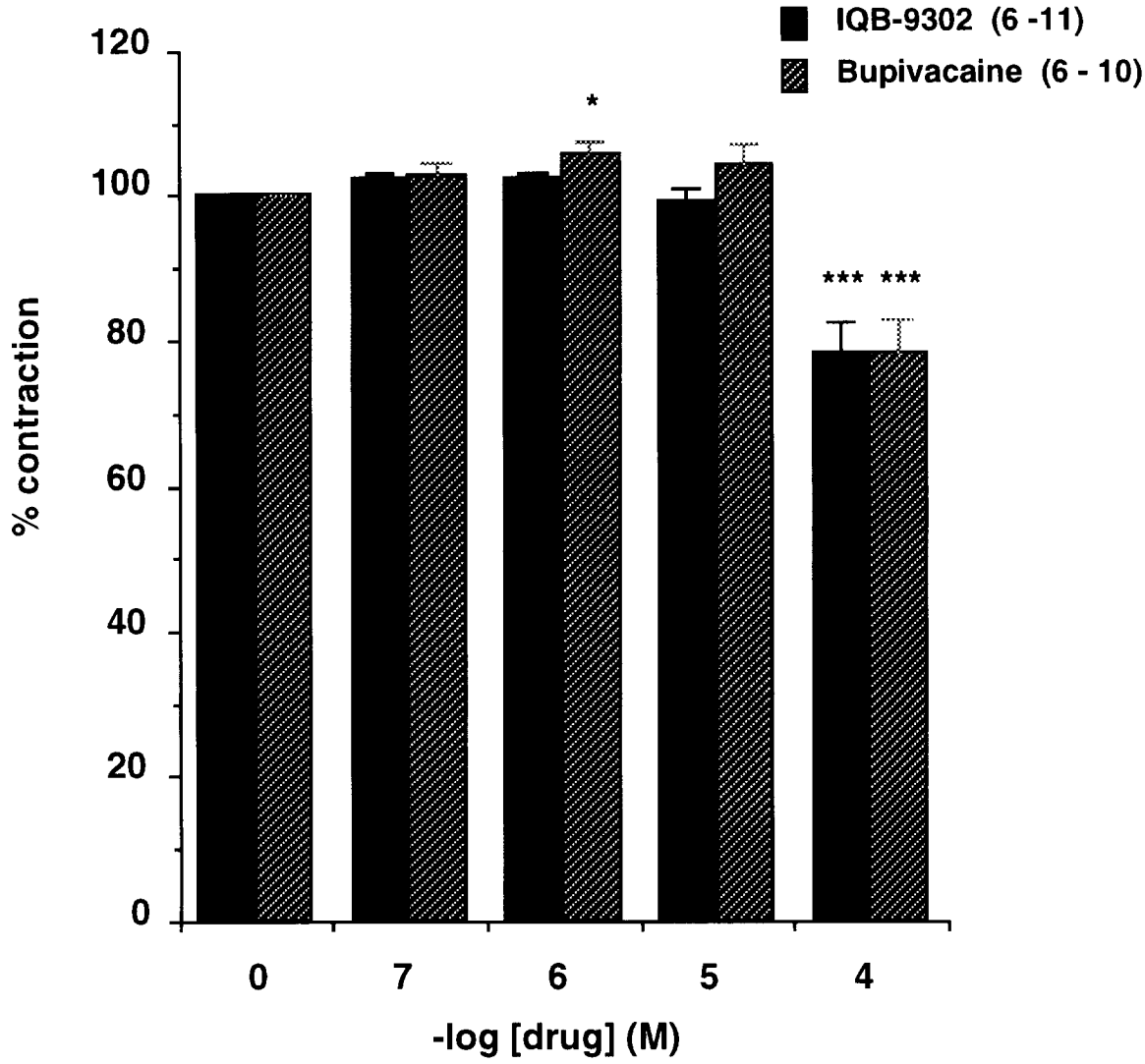

Fig. 3.- Effects of IQB-9302 (■) or bupivacaine (▨) on isolated rat aorta precontracted with 1 μM phenylephrine. The data correspond to means ± SEM of the number of experiments shown in parentheses. *P≤0.05, ***P≤0.001 with respect to their controls. (Calculations were made as in Fig. 2).

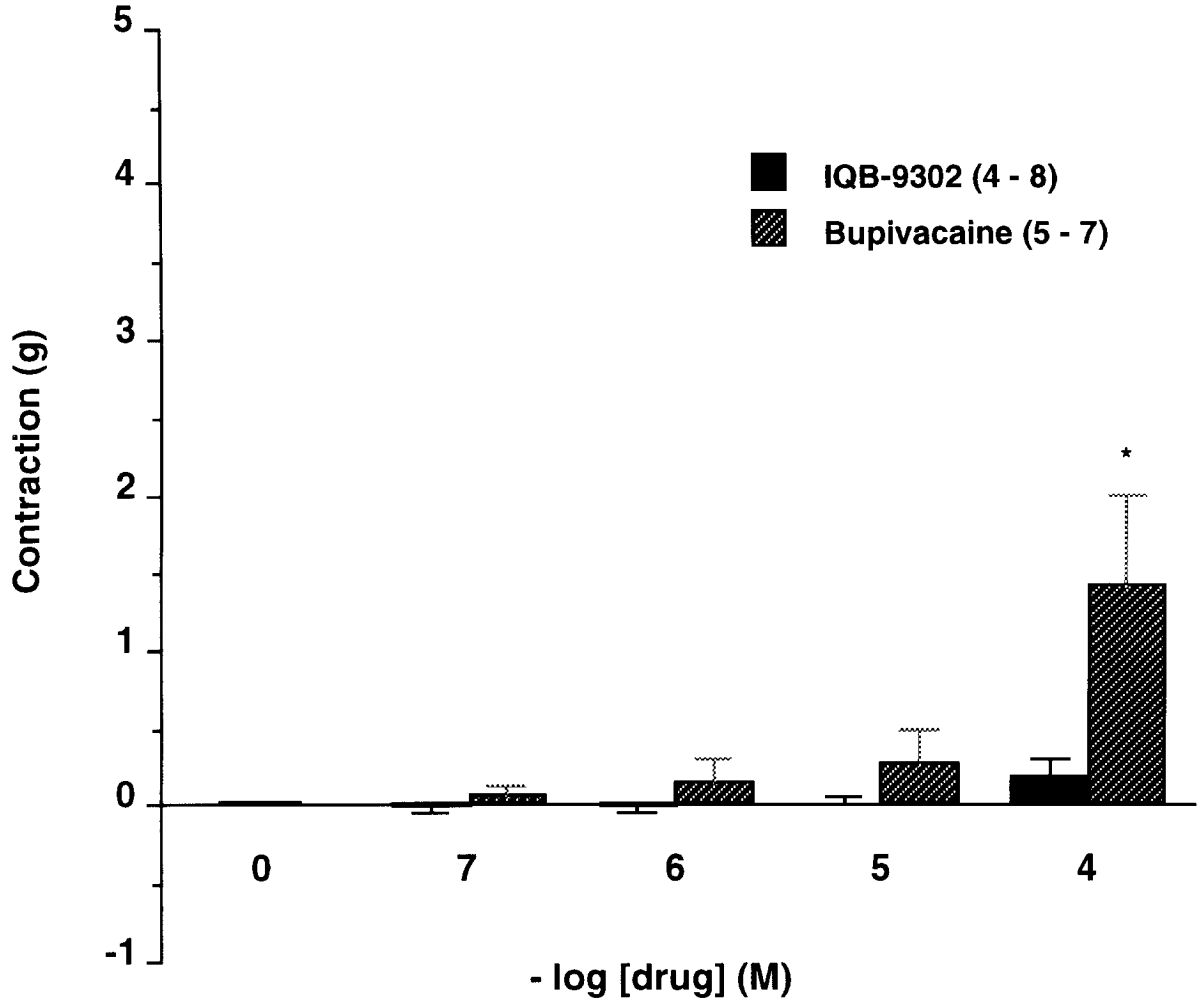

Fig. 4.-Effects of IQB-9302 (■) or bupivacaine (▨) on the basal tone of isolated rat aorta. The data correspond to the means ± SEM of the number of experiments shown in parentheses. *P≤0.05 with respect to control.