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Veratridine Augments and BDF 9148 Attenuates the Spontaneous Contractile Force of the Rat Portal Vein*

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Abstract

Veratridine and BDF 9148 both increase the force of myocardial contraction but their effects on the contractility of vascular smooth muscle are largely unknown. We have examined the effects of the drugs on the contractile force of the rat portal vein.

The force responses of the rat portal vein were not altered by tetrodotoxin (≤ 30 μM) but were augmented by Bay K 8644 (10 nM–10 μM), and attenuated by nifedipine (10 nM–10 μM). Veratridine slightly reduced the force of spontaneous contractions at concentrations of 0·1–1 μM, then augmented the force at concentrations up to 100 μM. The reduction was prevented by 30 nM tetrodotoxin; a higher concentration of 3 μM prevented the effect of veratridine. Pretreatment with nifedipine at 30 nM reduced the maximum augmentation observed with veratridine. BDF 9148 (0·3–300 μM) attenuated the force and this attenuation was increased in the presence of tetrodotoxin at 3 μM but reversed to an augmentation in the presence of Bay K 8644 at 10 μM. The augmentation produced by BDF 9148 in the presence of Bay K 8644 was reduced by tetrodotoxin at 30 nM.

These results suggest that veratridine predominantly opens sodium channels which in turn open voltage-dependent calcium channels to augment the force responses of the rat portal vein, but attenuate force in the presence of sodium-channel blockade with tetrodotoxin. BDF 9148 predominantly closes calcium channels to attenuate the force of the rat portal vein but in the presence of maximum calcium-channel opening with Bay K 8644 augments force probably by opening sodium channels.

The opening and closing of L-type calcium channels is associated with the constriction and dilation, respectively, of blood vessels. One blood vessel in which it is easiest to demonstrate calcium-channel modulation with drugs is the portal vein, where spontaneous contractile activity is augmented and attenuated by calcium-channel openers and blockers, respectively (Doggrell 1990). Vascular smooth muscle of various species has also been shown to contain sodium channels (Sturek & Hermsmeyer 1986; Okabe et al 1987; Mironneau et al 1990). The role, if any, of sodium channels in the contraction and relaxation of vascular smooth muscle is unknown.

Veratridine is a naturally occurring alkaloid that prolongs the cardiac action potential primarily by holding the activation gate of the sodium channel open (Honerjager 1982). BDF 9148 is a new drug that acts on cardiac tissue and reversibly prolongs the opening of sodium channels (Ravens et al 1991). Both compounds increase the force of myocardial contraction. The effects of veratridine and BDF 9148 on the contractility of vascular smooth muscle are largely unknown. The aim of this study was to characterize the effects of veratridine and BDF 9148 on a blood vessel.

Materials and Methods

Male Wistar rats were stunned and exsanguinated. The portal vein was removed and placed in Krebs solution that had been oxygenated with 5% CO_2 in O_2. All experiments were performed in the presence of modified Krebs solution (composition (mM): NaCl, 116; KCl, 5·4; $CaCl_2$, 2·5; $MgCl_2$, 1·2; NaH_2PO_4, 1·2; $NaHCO_3$, 22·0; D-glucose, 11·2; Na_2EDTA, 0·04) that was being oxygenated with 5% CO_2 in O_2 at 37°C. Contractile responses were recorded isometrically with force-displacement transducers (Grass model FTO3.C) and displayed on a polygraph (Grass model 79B). Experiments involving Bay K 8644 and nifedipine were performed in a dark room under orange photographic light to prevent drug degradation.

Four portal veins were studied simultaneously. Each was cleared of surrounding tissue and mounted longitudinally between two platinum electrodes under 1 g tension in 5 mL organ baths containing Krebs solution and left to equilibrate for 30 min. During the equilibration period the tissues were washed by overflow. The wash was then stopped and the tissues were left to stabilize for 20 min, during which time the spontaneous contractions became similar. Two tissues were then cumulatively challenged with tetrodotoxin, Bay K 8644, nifedipine, veratridine or BDF 9148 on an 8-min cycle while the other two tissues were challenged with the appropriate vehicle. Preliminary studies showed that the maximum effects of these drugs were obtained within 8 min. Some tissues were electrically stimulated (1 ms duration, supramaximal voltage) at 0·2–20 Hz for 2 min on an 8-min cycle in the absence or presence of tetrodotoxin.

In some experiments, after the equilibration period, tissues were treated with phenoxybenzamine (50 μM) for 30 min to block α-adrenoceptors and then washed by rapid overflow for 30 min before being left to stabilize for 20 min before challenge with drugs or vehicle. When studying the effect of a drug in the presence of tetrodotoxin, Bay K 8644, or nifedipine, either together or in combination, the tissues were treated with Bay K 8644 or nifedipine for 30 min before challenge with the drug or its vehicle.

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*A preliminary account of this study has been presented to the British Pharmacological Society (Doggrell & Bishop 1994).

Assessment of data

The amplitudes of the final three contractions before addition of the first or higher concentrations of a drug were measured and averaged. The amplitude before the addition of drug was denoted 100%. In untreated tissues the force of contractions increased with time such that, typically, the force was $125 \pm 6\%$ (n = 8) in these tissues at the end of the challenge with drug. The force of contractions in the presence of single drugs was calculated as a percentage difference from the appropriate time control. Vehicles of HCl at $\leq 10^{-3}$ M or ethanol at $\leq 0{\cdot}02\%$ had no effect on the force of contractions of the portal vein. Ethanol at $> 0{\cdot}02\%$ attenuated the contractions, however. The effects of high concentrations of BDF 9148 (3×10^{-5} M) in $> 0{\cdot}02\%$ ethanol were calculated as a percentage change from the ethanol-treated tissue run in parallel. The force of contraction of one drug in the presence of another, drug A, was calculated as a percentage difference of the contractions in the presence of A at the appropriate time. For drugs that augmented or attenuated the force responses the EC50 and IC50 values, i.e. the molar concentrations of the drugs that caused 50% of the maximum augmentation or attenuation, respectively, were determined by linear regression.

Drugs

The drugs used were (±)-Bay K 8644 (donated by Bayer), BDF 9148 (4-[3-(1-diphenylmethylacetidin-3-oxy)-2-hydroxypropoxy]-1*H*-indol-2-carbonitrile; donated by Beiersdorf AG), phenoxybenzamine hydrochloride (Research Biochemicals Incorporated), nifedipine, tetrodotoxin and veratridine (Sigma). The stock solutions were veratridine (100 mM in 100 mM HCl), Bay K 8644, BDF 9148 and nifedipine (each 10 mM in absolute ethanol) and phenoxybenzamine (100 mM in absolute ethanol containing 10 mM HCl. All dilutions of these drugs were in distilled water.

Results

The rat portal vein has spontaneous contractile activity (Fig. 1).

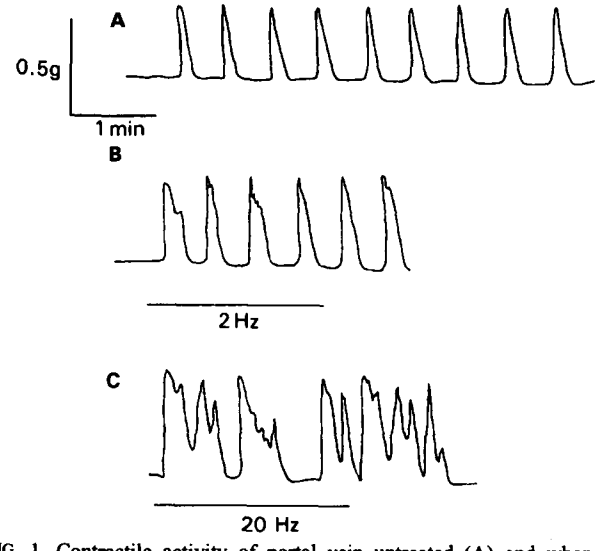

FIG. 1. Contractile activity of portal vein untreated (A) and when electrically stimulated at 2 (B) or 20 (C) Hz.

Effects of tetrodotoxin, Bay K 8644 and nifedipine

Tetrodotoxin, 0·1–30 μM, alone had no effect on portal vein contractile activity (n = 4, data not shown). Electrical stimulation (1 ms duration, supramaximal voltage) at 0·2–20 Hz for 2 min had no effect or a small augmenting effect on the force of responses and at the higher frequencies of stimulation, 2–20 Hz also caused prolongation of the duration of the contractions (Fig. 1). Tetrodotoxin at 30 nM abolished the electrical-stimulation-induced prolongation of contractions (n = 4, data not shown).

Bay K 8644 and nifedipine at 10 nM–10 μM augmented and attenuated the force responses (Fig. 2); EC50 and IC50 values were $0{\cdot}30 \pm 0{\cdot}04$ μM (n = 4) and $0{\cdot}65 \pm 0{\cdot}26$ μM (n = 6), respectively. In the presence of Bay K 8644 at 10 μM, nife-

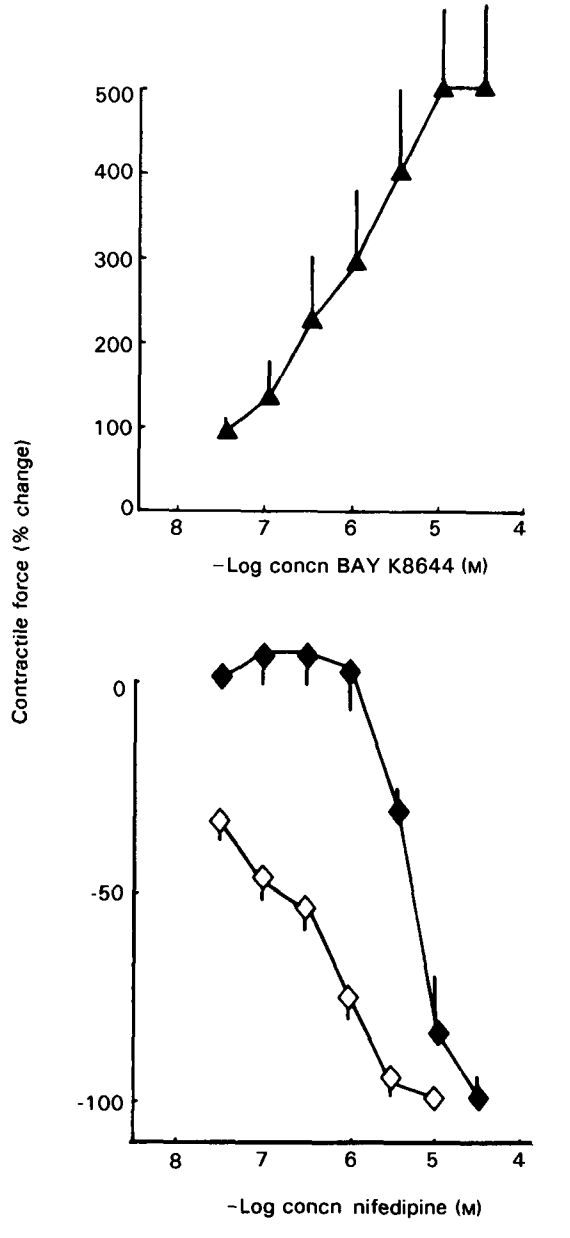

FIG. 2. The effects of Bay K 8644 and nifedipine on the contractile force of the rat portal vein. Top, the effect of Bay K 8644 alone (▲); bottom, the effect of nifedipine alone (◇) and in the presence of Bay K 8644 at 10 μM (◆). Each value is the mean ± s.e.m. (n = 3–6).

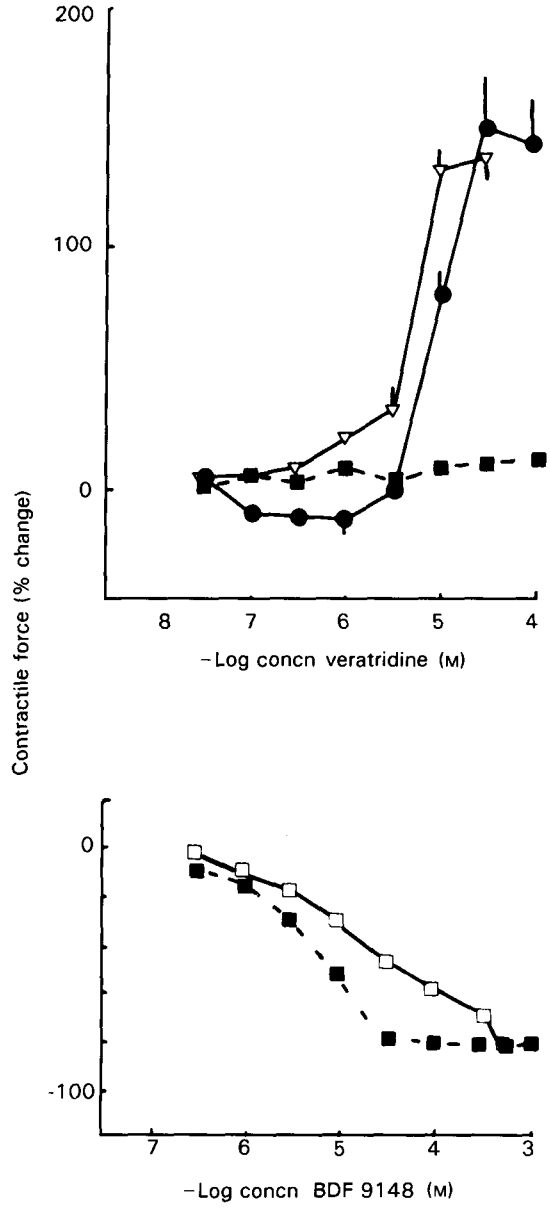

FIG. 3. The effects of veratridine (top) and BDF 9148 (bottom) on the contractile force of the rat portal vein. Top, the effect of veratridine alone (●) and in the presence of tetrodotoxin at 30 nM (▽) or 3 μM (■); bottom, the effect of BDF 9148 alone (□) and in the presence of tetrodotoxin at 3 μM (■). Each value is the mean ± s.e.m. (n = 4–11).

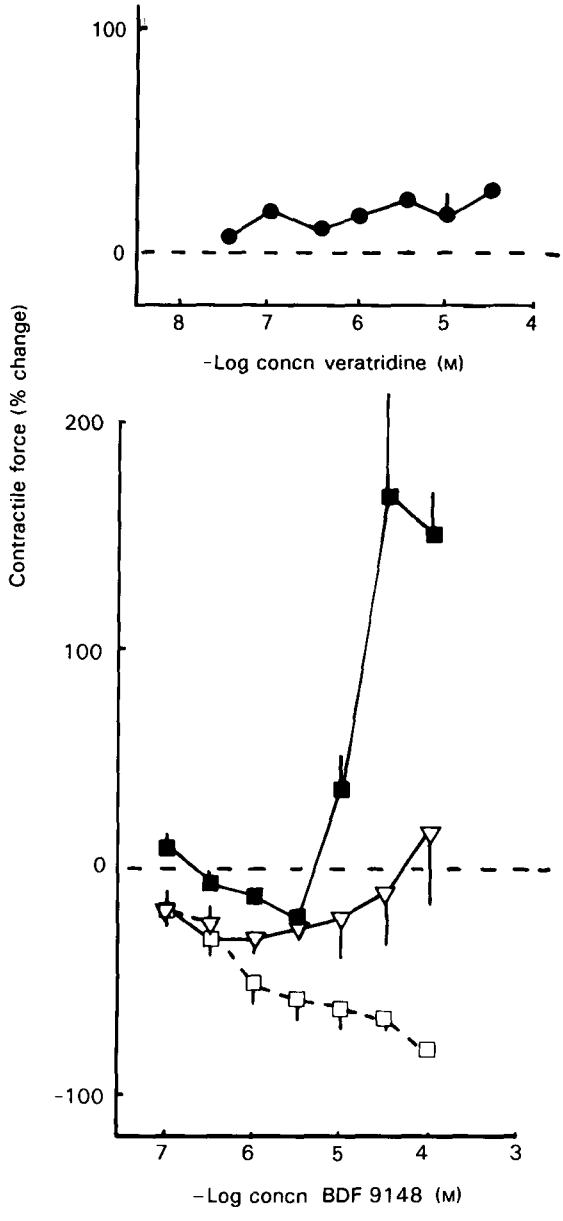

FIG. 4. The effects of veratridine (top) and BDF 9148 (bottom) in the presence of Bay K 8644. Top, the effect of veratridine in the presence of Bay K 8644 at 10 μM (●); bottom, the effect of BDF 9148 in the presence of Bay K 8644 (■) and in the additional presence of tetrodotoxin at 30 nM (▽) or 3 μM (□). Each value is the mean ± s.e.m. (n = 4–6).

dipine at ≤ 1 μM had no effect and at 3–30 μM attenuated the force responses (Fig. 2) with an IC50 of 3·2 ± 3·5 μM (n = 3).

Effects of veratridine and BDF 9148 alone

Veratridine at 0·1–1 μM caused a small significant attenuation ($P < 0.05$), at 3 μM had no effect, and at 10–100 μM augmented the force of the contractile activity (Fig. 3) with an augmentation EC50 of 6·8 ± 1·1 μM (n = 11). BDF 9148 at 0·3–1 μM had no effect but at 3–300 μM attenuated the force with an IC50 of 25 ± 14 μM (n = 6), Fig. 3. None of the effects of veratridine and of BDF 9148 was altered by pretreatment of the veins with phenoxybenzamine (50 μM for 30 min, n = 4, data not shown).

Effects of veratridine in the presence of tetrodotoxin, Bay K 8644 or nifedipine

The ability of low concentrations of veratridine to attenuate the force was abolished in the presence of tetrodotoxin, 30 nM or 3 μM (Fig. 3). Only tetrodotoxin at 3 μM (not 30 nM) abolished the augmenting action of high concentrations of veratridine, however (Fig. 3). In the presence of maximum augmentation to Bay K 8644 at 10 μM, veratridine 0·1–30 μM caused a further small significant augmentation, $P < 0.05$ (Fig. 4). Treatment with nifedipine at 30 nM for 30 min attenuated the contractile force by 50 ± 4% (n = 9). In the presence of nifedipine at 30 nM the attenuating effects of veratridine at 0·1 and 0·3 μM

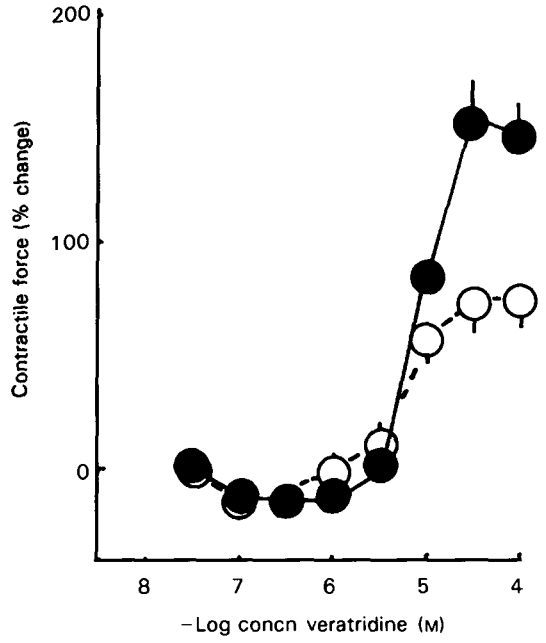

FIG. 5. The effects of veratridine in the absence (●) and in the presence (○) of nifedipine (3×10^{-8} M). Each value is the mean ± s.e.m. (n = 9–11).

were unaltered (Fig. 5). The augmenting effects of veratridine at 10–100 μM were, however, reduced in the presence of nifedipine (Fig. 5).

Effects of BDF 9148 in the presence of tetrodotoxin or Bay K 8644

The ability of BDF 9148 to attenuate the contractile force of the portal vein was not altered by tetrodotoxin at 30 nM (n = 4, data not shown) but was significantly increased, $P < 0.05$, in the presence of tetrodotoxin at 3 μM (Fig. 3). In the presence of Bay K 8644 at 10 μM, the lowest concentrations of BDF 9148 (1–3 μM) continued to attenuate the force whereas the higher concentrations of BDF 9148 (10–100 μM) augmented the force responses. This augmentation produced by BDF 9148 in the presence of Bay K 8644 was reduced by tetrodotoxin at 30 nM and reversed to an attenuating action by tetrodotoxin at 3 μM (Fig. 4).

Discussion

Several subtypes of sodium channel exist; some are tetrodotoxin-sensitive whereas others are not (Grant & Wendt 1992). Tetrodotoxin alone had no effect on the spontaneous contractile activity of the portal vein, which suggests that sodium-channel opening is not involved in the spontaneous activity and also that tetrodotoxin ≤ 30 μM has no non-specific effects on the portal vein. Electrical stimulation of the rat portal vein at frequencies of 2 Hz and above caused prolongation of the relaxation phase of the contractions, presumably by initiating transmitter-release from nerves (e.g. noradrenaline from noradrenergic nerves). A low concentration of tetrodotoxin, 30 nM, prevented this prolongation, presumably by blocking a tetrodotoxin-sensitive sodium channel associated with the nerve activity.

The spontaneous contractile activity of the portal vein is associated with the opening and closing of calcium channels (Sutter 1990). Bay K 8644 and nifedipine are drugs the predominant actions of which are, respectively, to open and close calcium channels. In this study, Bay K 8644 and nifedipine acted as expected to augment and attenuate, respectively, the contractile force of the rat portal vein. In addition the sensitivity to nifedipine was reduced in the presence of Bay K 8644.

The primary aim of this study was to characterize the effects of veratridine and BDF 9148 on a blood vessel. Activation of the noradrenergic nerves of rat portal vein releases noradrenaline to stimulate α-adrenoceptors and augment contractile activity; this effect is blocked by phenoxybenzamine. In this study phenoxybenzamine did not modify the actions of veratridine or BDF 9148 on the portal vein. Thus neither of these drugs directly or indirectly stimulates portal vein α-adrenoceptors. We have indirect evidence that veratridine and BDF 9148 may be interacting with sodium and calcium channels on the rat portal vein with veratridine probably acting predominantly to open sodium channels to cause augmentation, whereas BDF 9148 probably acts predominantly to close calcium channels to produce attenuation of the spontaneous contractile activity. Thus, low concentrations of veratridine attenuated the contractile force by opening a sodium channel that was blocked by a low concentration of tetrodotoxin, and high concentrations of veratridine augmented the force by opening a sodium channel that was blocked by a high concentration of tetrodotoxin. The augmenting effect of veratridine is also reduced in the presence of nifedipine, presumably because the prolongation of the opening of the sodium channels with veratridine initiates the opening of voltage-dependent calcium channels, Ca^{2+} entry into the cells and the increase in contractile force. The location of the tetrodotoxin-sensitive and tetrodotoxin-insensitive sodium channels remains to be determined.

The most interesting and unexpected finding of this study was that BDF 9148, which hitherto has been considered to be predominantly a sodium-channel opener, attenuated the force responses of the portal vein. Our preliminary studies have also shown that BDF 9148 relaxes rat aortae that have been contracted with KCl. A number of mechanisms could underlie the ability of BDF 9148 to attenuate the force and relax blood vessels. A previous electrophysiology study (Ravens et al 1991) has shown that BDF 9148 blocks calcium channels in cardiac cells. Thus it is possible that BDF 9148 is relaxing the portal vein by blocking calcium channels. The attenuating effects of BDF 9148 and nifedipine were reduced by the presence of Bay K 8644 which is supportive evidence that BDF 9148 may be blocking the portal vein calcium channels. In the presence of Bay K 8644, high concentrations of BDF 9148 caused a small augmentation, which was probably a result of the opening of sodium channels, as the effect was reversed by tetrodotoxin.

Positive inotropes and vasodilators are among the standard drugs used in the treatment of heart failure. BDF 9148 is being developed as a positive inotrope for treatment of heart failure (Hoey et al 1993). The present study suggests that the same concentrations of BDF 9148 that open cardiac sodium channels to cause a positive inotropic effect are also closing vascular calcium channels to initiate relaxation of the portal vein. The effects of BDF 9148 on other blood vessels requires exam-

ination. The ability of BDF 9148 to act as a positive inotrope and to cause some dilation of blood vessels provides this drug with potential for the treatment of heart failure.

Acknowledgement

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References

Doggrell, S. A. (1990) The assessment of the β_2-adrenoceptor and Ca^{2+} channel blocking activity of drugs with the rat portal vein. J. Pharmacol. Methods 24: 145–156

Doggrell, S. A., Bishop, B. E. (1994) The effects of veratridine and BDF 9148 on the contractile force of the rat isolated portal vein. Br. J. Pharmacol. 112: 199P

Grant, A. O., Wendt, D. J. (1992) Blockade of modulation of cardiac Na^+ channels by antiarrhythmic drugs, neurotransmitters and hormones. TIPS 13: 352–358

Hoey, A., Lehmkuhl, A., Sadony, V., Ravens, U. (1993) Inotropic actions of BDF 9148 and DPI 201-106 and their enantiomers in guinea-pig, rat and human atria. Eur. J. Pharmacol. 23: 477–479

Honerjager, P. (1982) Cardioactive substances that prolong the open state of sodium channels. Rev. Physiol. Biochem. Pharmacol. 82: 1–74

Mironneau, J., Martin, C., Arnaudeau, S., Jmari, K., Rakotoariso, L., Sayet, I., Mironneau, C. (1990) High affinity binding sites for [^{3}H]-saxitoxin are associated with voltage-dependent sodium channels in portal vein smooth muscle. Eur. J. Pharmacol. 184: 315–319

Okabe, K., Kitamura, K., Kuriyama, H. (1987) Features of 4-aminopyridine sensitive outward current observed in single smooth muscle cells from the rabbit pulmonary artery. Pflügers Arch. 409: 561–568

Ravens, U., Wettwer, E., Pfeifer, T., Himmel, H., Armah, B. (1991) Characterization of the effects of the new inotropic agent BDF 9148 in isolated papillary muscles and myocytes of the guinea-pig heart. Br. J. Pharmacol. 104: 1019–1023

Sturek, M., Hermsmeyer, K. (1986) Calcium and sodium channels in spontaneously contracting vascular smooth cells. Science 233: 475–478

Sutter, M. C. (1990) The mesenteric portal vein in research. Pharmacol. Rev. 42: 287–325