substantial reduction, the order of potency being BW755C > tamoxifen > flurbiprofen.

Discussion

The human peripheral blood PMNs stimulated with the calcium ionophore A23187 converted [^{14}C]arachidonic acid to LTB_4 and 5-HETE; this conversion was concentration-relatedly inhibited by the lipoxygenase/cyclooxygenase inhibitor BW755C (Salmon et al 1983). At the highest concentration (100 $\mu g\,mL^{-1}$) tamoxifen citrate and flurbiprofen also caused substantial inhibition, and a similar weak trend was seen with 10 $\mu g\,mL^{-1}$. Daniel et al (1981) found a mean tumour content of 25·1 (range 5·4–117) ng tamoxifen mg^{-1} protein in patients taking 40 mg tamoxifen daily. Patterson et al (1982) converted the mean value to 6·7 μM (2·5 $\mu g\,mL^{-1}$) by assuming a 10% content of protein in the tumours, and on the same basis the top of the range would be 11·7 $\mu g\,mL^{-1}$ tamoxifen (equivalent to 17·7 $\mu g\,mL^{-1}$ tamoxifen citrate). Thus an effect on 5-lipoxygenase may occur at therapeutically relevant concentrations. Furthermore, preliminary evidence indicates that the 5-lipoxygenase in a cell-free system is more sensitive than human PMNs to tamoxifen. This may be due to the rather high resistance of human PMNs to drugs that modify eicosanoid synthesis (Tavares et al 1986). It remains to be seen whether inhibition of 5-lipoxygenase contributes to the beneficial effect of tamoxifen in breast cancer.

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Potentiation of contractile response and increase in tissue sodium content induced by aconitine in the guinea-pig vas deferens

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Aconitine potentiated the contractile response of the guinea-pig vas deferens and increased the tissue Na and Ca content. These effects were abolished in the presence of tetrodotoxin. These results suggest that aconitine causes an increasing Na^+ permeability of the smooth muscle membrane to increase Ca^{2+} availability and thus induces potentiation.

In Japan and China, *Aconitum* root has been used to treat symptoms such as pain, paralysis, atonia and coldness of extremities and one of the alkaloids from the root, aconitine, is known to produce an arrhythmia of the heart (Scherf & Terranova 1949). Electrophysiological studies have revealed that aconitine-induced arrhythmia is due to a delay in the repolarization phase of the action potential (Matsuda et al 1959; Schmidt 1960). In frog skeletal muscles, aconitine causes bursts of repetitive firing which are prevented by tetrodotoxin (TTX) (Ellis & Bryant 1973). Aconitine has been shown to induce an increase in the membrane permeability of the squid giant axon (Herzog et al 1964) and motor nerve fibres (Schmidt & Schmitt 1974) to Na^+. Mesaconitine, one of the related compounds of aconitine has been found to cause a marked contraction of the guinea-pig isolated vas deferens (Sato et al 1979) and ileum (Sato et al 1980) mediated through neurotransmitter release. However, the direct actions of these alkaloids on smooth muscles have not yet been studied. In this paper, we report the first evidence of the direct action of aconitine on smooth muscle using guinea-pig vas deferens.

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Methods

Male guinea-pigs, 250 to 350 g, were used. The method of preparing the tissue and the technique for measurement of contractions were performed as described

previously (Ohizumi & Shibata 1980). The tissues were suspended in Krebs-Ringer solution of the following composition (mM): NaCl 120, KCl 4·8, $CaCl_2$ 1·2, $MgSO_4$ 1·3, KH_2PO_4 1·2, $NaHCO_3$ 25·2 and glucose 5·8, pH 7·4. The 20 mL organ bath containing Krebs-Ringer solution was bubbled with 95% O_2–5% CO_2. The isometric tension changes were measured by a force displacement transducer. The activity of Na^+,K^+-ATPase (Sigma) isolated from porcine cerebral cortex was assayed as described previously (Ohizumi et al 1982). To measure the tissue Na, K and Ca content, the vas deferens was ashed as described by Ohizumi et al (1982, 1983). The amount of ions was determined using an atomic absorption spectrophotometer. In the present experiment the Na and K content was measured after washing the vas deferens with cold Li-substituted Krebs-Ringer solution for 15 min to remove extracellular Na. For cold storage preparations, the vas deferens was stored at 4 °C for 7 days. The procedure leads to the degeneration of intrinsic nerves in smooth muscle preparations (Ambache 1946; Lum et al 1966; Varma & McCullough 1969). The cold-stored preparations were preincubated at 32 °C in the solution for 2 h before the application of drugs. The following drugs were used: aconitine (Sigma), noradrenaline bitartrate (Sigma), tetrodotoxin (Sankyo) and ouabain (Merck). Aconitine was dissolved in ethanol of which the final concentration was kept of 0·03% and other drugs were dissolved in distilled water.

Results

Aconitine (4×10^{-5} M) induced only small contractions of the tissue, but, after treatment with aconitine, the dose-response curve for noradrenaline (NA) or KCl was markedly shifted to the left, indicating potentiation (Fig. 1). As shown in Table 1, contractile responses to NA (3×10^{-6} M) and KCl (2×10^{-2} M) were potentiated by 25 and 22%, respectively, in the presence of aconitine. The aconitine-induced potentiation of NA and KCl was abolished by a specific Na channel blocker, tetrodotoxin (TTX, 10^{-7} M) (Table 1). In the cold-stored preparations, aconitine at 4×10^{-5} and 10^{-4} M, did not affect the resting tension, but was still able to potentiate the NA (3×10^{-6} M)-induced contraction from 1·5 ± 0·2 (n = 6) to 1·8 ± 0·1 (n = 6 or by 20%, and 2·2 ± 0·2 g (n = 6) or by 47%, respectively. After washing with fresh medium, the contractile response to NA was further increased to 3·3 ± 0·2 (n = 6) (by 120%) and 3·6 ± 0·2 g (n = 6) (by 140%), respectively.

Aconitine (4×10^{-5} M) alone had little or no effect on the tissue Na content of the vas deferens. However, as shown in Table 1, in the presence of ouabain (10^{-5} M), a Na^+,K^+-pump inhibitor, the tissue Na content was increased 39% by aconitine 30 min after the application. On the other hand, the K content was decreased by 16%. These effects of aconitine were completely inhibited by treatment with TTX (10^{-6} M) (Table 1). The Na and K contents were further elevated (by 48%) and reduced (by 25%), respectively, 30 min after washing with the medium without aconitine. In addition, aconitine also caused an increase in the tissue Ca content (by 17%), which was abolished by treatment with TTX (10^{-6} M). The effect of aconitine on Na^+,K^+-ATPase purified from porcine cerebral cortex was

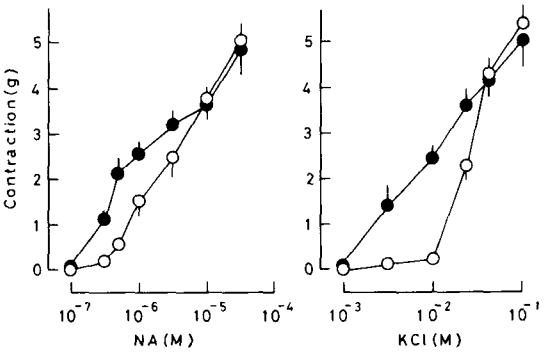

FIG. 1. Effects of aconitine on the dose-response curve for noradrenaline (NA) and KCl in the guinea-pig vas deferens, ○, control; ●, aconitine (4×10^{-5} M). NA or KCl was added 30 min after application of aconitine. Each dose-response curve was obtained by using different tissues. The responses are expressed as the mean ± s.e.m. of 5 experiments.

Table 1. Effects of aconitine (4×10^{-5} M) on the noradrenaline (NA)- and KCl-induced contraction and the tissue Na, K and Ca content of the guinea-pig vas deferens in the presence or absence of tetrodotoxin (TTX). TTX (10^{-7} M for tension experiments, 10^{-6} M for the ion content experiments) was added 15 min before the application of aconitine. The contractile response to agonists was measured 30 min after the application of aconitine. For Na and K content experiments, each vas deferens was incubated with the solution containing aconitine for 30 min and then was washed with the Na^+-free Li^+ solution for 15 min and the ion content experiment was done in the presence of ouabain (10^{-5} M) to block the Na^+,K^+-pump.

	Tension (g)		Tissue content (μmol g^{-1} wet wt)		
Treatment	Na (3×10^{-6} M)	KCl (2×10^{-2})	Na	K	Ca
None	1·9 ± 0·2	1·7 ± 0·2	23·1 ± 1·9	59·8 ± 1·5	0·761 ± 0·03
Aconitine	2·9 ± 0·2*	3·0 ± 0·2*	32·2 ± 0·7*	50·5 ± 0·8*	0·890 ± 0·02*
Aconitine + TTX	2·1 ± 0·3	1·2 ± 0·2	22·7 ± 1·1	57·4 ± 2·0	0·750 ± 0·03

* Significantly different from control (none) ($P < 0·05$). Results are given as means ± s.e.m. of 8 preparations.

examined. Aconitine (10^{-5} to 10^{-4} M) had little effect on the activity at any of the concentrations used.

Discussion

In the guinea-pig isolated vas deferens, aconitine induced a potentiation of the contractile response to NA and KCl. After cold-storage, a procedure which leads to loss of physiological nerve functions (Ambache 1946; Lum et al 1966; Varma & McCullough 1969), aconitine was still capable of producing a marked potentiation of the NA-induced contraction. Furthermore, treatment of guinea-pig with reserpine (1 mg kg^{-1} day^{-1}, for 2 days), a catecholamine-depleting agent, had no or little effect on the aconitine-induced potentiation of the response to Na or KCl (unpublished data). These data suggest that the potentiation by aconitine may be largely due to an increase in excitability of the postsynaptic membrane of the vas deferens. It has been previously reported that ciguatoxin, a potent depolarizing agent, induced a postsynaptic supersensitivity in the guinea-pig vas deferens, with pharmacological properties similar to those induced by aconitine (Ohizumi et al 1982).

It has been indicated that aconitine causes a membrane depolarization of skeletal muscles and motor nerve fibres, which is blocked by TTX (Ellis & Bryant 1973; Schmidt & Schmitt 1974). In cardiac muscle, the positive inotropic and electrophysiological effects of aconitine have been reported to be abolished by TTX (Honerjäger & Meissner 1983). Therefore, these excitatory effects of aconitine on skeletal and cardiac muscles and nerves are explained by activation of TTX-sensitive Na channels. In the present experiments, aconitine produces a potentiation of the contraction induced by agonists which is accompanied by an increase in the tissue Na and Ca content. These effects of aconitine were antagonized by TTX. In addition, aconitine was without effect on Na^+,K^+-ATPase activity, indicating that possible involvement of inhibition of the Na^+,K^+-pump by aconitine was ruled out. It is widely accepted that in smooth muscle Na^+ may directly control the intracellular Ca^{2+} concentration through a Na-Ca exchange mechanism (Blaustein 1977; Van Breemen et al 1979). These studies on the Na-Ca exchange mechanisms have shown that an increased concentration of intracellular Na^+ causes an increase in Ca^{2+} influx and a decrease in Ca^{2+} efflux and thus raises intracellular Ca^{2+} concentrations (Van Breemen et al 1979). On the basis of these observations, it is suggested that aconitine probably activates the TTX-sensitive Na channels of smooth muscle cells and this may result in an increase in permeability of the smooth muscle cell membrane to Ca^{2+} so that a potentiation in the vas deferens results from treatment with aconitine.

In the presence of ouabain, aconitine caused an increase in the tissue Na content of the vas deferens. But, in the absence of ouabain the Na content was not changed by aconitine. Numerous studies have suggested that the elevated intracellular Na^+ concentration stimulates the Na^+,K^+-pump activity in many tissues. Therefore, it is possible that the elevation of intracellular Na^+ concentration by aconitine is cancelled by activation of the Na^+,K^+-pump in the absence of ouabain. This may be a reason for the lack of effects of aconitine alone on the tissue Na content.

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