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The excitatory effect of dopamine on isolated canine tracheal smooth muscle

CHUNG-HUNG SHUE*, CHING-JU CHEN, *Department of Physiology, *Institute of Clinical Medicine, National Yang-Ming Medical College, Taipei, Taiwan*

Abstract—The effect of exogenous dopamine on canine tracheal smooth muscle has been studied in-vitro. Dopamine at concentrations over 10^{-5} M induced contractions of tracheal muscle strips and repeated exposures resulted in desensitization (tachyphylaxis) of the muscle. The sensitivity of the response varied dramatically among muscle strips. At lower concentrations, dopamine caused neither muscle relaxation nor inhibition of contractions evoked by 10^{-6} M acetylcholine. Both a dopaminergic antagonist, haloperidol (10^{-5} and 10^{-4} M), and an α-adrenoceptor antagonist, phentolamine (10^{-7} to 10^{-5} M), attenuated the contraction to 10^{-3} M dopamine. The β-adrenoceptor antagonist, propranolol (10^{-8} to 10^{-6} M), enhanced the contraction. However, the contraction could only be abolished by phentolamine at 10^{-4} M. Thus, in canine tracheal smooth muscle, the contractile response to dopamine is predominantly through the activity of α-adrenoceptors and the role of dopaminergic receptors is vague. It is suggested that the weakness of the dopamine-induced contraction results from an antagonism between α- and β-adrenoceptor effects and the dopamine tachyphylaxis may reflect a gradually decreased activation of the α-adrenoceptor mechanism in comparison with the β-adrenoceptor mechanism.

Desensitization (tachyphylaxis) is a frequently observed phenomenon in which repeated exposure of a tissue to an agent decreases the response to that agent. Several in-vitro studies have described the tachyphylaxis of airway smooth muscle as a result of repeated challenges with histamine (Anderson et al 1979; Brink et al 1982; Shore et al 1983; Antol et al 1988). Dopamine-induced tachyphylaxis has been reported only by Advenier et al (1980) in their study of the guinea-pig isolated trachea.

This report describes the response of canine isolated tracheal smooth muscle strips to dopamine and the development of dopamine tachyphylaxis after repeated challenges.

Materials and methods

Method. Mongrel dogs were killed with pentobarbitone sodium (30 mg kg^{-1}) and a portion of the extrathoracic trachea consisting of 6 rings was removed, rinsed and transported in Krebs solution (mM: NaCl 118, KCl 4·5, $CaCl_2$ 2·5, $MgSO_4$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 25·5 and glucose 5·6) at 4°C. About one third of the tracheae used were from animals killed after in-vivo airway resistance studies. These specimens were stored overnight in Krebs at 4°C. The posterior membranous region of the isolated trachea was dissected free of epithelium and loose connective tissue and the smooth muscle was cut transversely into strips 3 mm wide and 15 mm long. Each strip was then mounted vertically in an organ bath with 50 mL of Krebs at 37°C aerated with 95% O_2 and 5% CO_2.

Isometric tension of each strip was monitored with a Grass model FT.03 force displacement transducer connected to a Grass model 7D polygraph. After being stretched to a passive tension of 2 g, the strip was equilibrated for 2 h in the bath. Resting tension was then adjusted to 2 g again before the start of the experiment.

The concentration effect curves were produced by challenging a group of 18 strips with dopamine at increasing concentrations (10^{-9} to 6×10^{-3} M). Dopamine was added to the bath and the bath fluid was replaced with fresh Krebs solution after the contraction had reached a plateau. If there was no contraction, the bath fluid was replaced after 10 min. The strips were washed twice more in 15 min before dopamine of a higher concentration was added. Another group of 18 muscle strips was used to study the possible muscle relaxing effect of dopamine. Strips were pretreated for 5 min with dopamine (10^{-10} to 10^{-3} M) and challenged with 10^{-6} M acetylcholine (ACh) in the continued presence of dopamine.

The response of canine tracheal smooth muscle to repeated challenges of dopamine was examined by exposing 18 strips to 10^{-3} M dopamine 5 or 6 times and washing the strips with 3 changes of fresh Krebs solution between exposures. Once the response to dopamine became too weak to be observed, some of the strips were given repeated challenges of 10^{-6} M acetylcholine and others were given repeated challenges of 30 mM KCl to confirm their ability to contract. In parallel experiments, the interval between two dopamine challenges was extended to 30 min, 1 h and 3 h.

The effects of the dopaminergic antagonist, haloperidol and the adrenoceptor antagonists, phentolamine and propranolol, on the dopamine-induced contraction were investigated by pretreating muscle strips with an antagonist for 5 min and challenging these strips with 10^{-3} M dopamine in the continued presence of the antagonist. This pretreatment-challenge process was repeated four times. The concentration-dependency of the antagonistic effect was determined using different groups of 4–6 strips from different animals. In some experiments with haloperidol, the duration of pretreatment was extended to 20 min.

Drugs. Dopamine (Kali-Chemie Pharma GmbH), acetylcholine chloride (Sigma), potassium chloride (Sigma), haloperidol (Sigma), phentolamine (Regitine, Ciba-Geigy) and (±)-propranolol hydrochloride (Sigma) were diluted with Krebs solution to 100 times the desired concentration immediately before the experiment so that the final dilution could be reached by replacing 0·5 mL of the bathing solution with the diluted drug stock solution. Ascorbic acid, (1·1 mM) was added to the catecholamine solutions to prevent oxidation.

Since, in canine tracheal smooth muscle, the amplitude of response to dopamine varied among muscle strips and the contractions to repeated challenges of dopamine decreased progressively after the second contraction, its amplitude was designated as 100% (maximal) contraction and subsequent contractions were expressed as % of the maximal contraction. Statistical analysis was by two-way ANOVA and Tukey's range test. A P value smaller than 0·05 was considered significant.

Results

About 80% of the strips used gave a contractile response to dopamine, although the sensitivity varied dramatically among different strips from the same or different animals. Tachyphylaxis occurred in all responsive strips after repeated challenges and a time-dependent reversal of tachyphylaxis was not observed. Since the contractile responses of tracheal muscle strips from animals killed after in-vivo experiments showed no statistical

Correspondence to: C.-J. Chen, Department of Physiology, National Yang-Ming Medical College, Shih-pai, Taipei 11221, Taiwan.

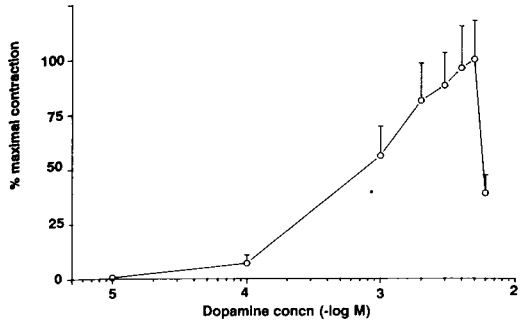

FIG. 1. Concentration-effect curve of canine tracheal smooth muscle strips exposed to dopamine. Data are expressed as mean ± s.e. (n = 18). The response began at 10^{-4} M. The EC50 is close to 10^{-3} M and the maximal response occurred at 5×10^{-3} M.

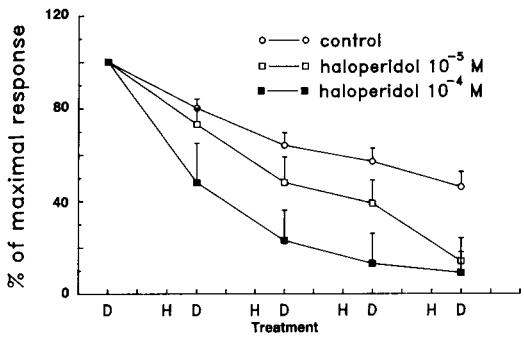

FIG. 2. Concentration-dependent inhibitory effect of a dopaminergic antagonist, haloperidol (H), on contractions induced by repeated challenges of 10^{-3} M dopamine (D). Data are expressed as mean ± s.e. (n = 18 for control, n = 6 for each concentration of haloperidol). Among the concentrations tested, 10^{-9}–10^{-4} M, only the highest two produced significant inhibition of dopamine-induced contraction ($P < 0.05$).

difference from those of strips prepared solely for in-vitro experiments, data from both sources were pooled.

The tracheal muscle strips developed contraction to dopamine when the concentration was raised to 10^{-4} M. The maximal contraction was reached at 5×10^{-3} M (Fig. 1). The EC50 of dopamine was close to 10^{-3} M. At concentrations between 10^{-10} and 10^{-5} M, dopamine brought about neither relaxation nor inhibition of contractions evoked by 10^{-6} M acetylcholine (data not shown). At 10^{-4} and 10^{-3} M, a facilitatory effect of dopamine was statistically significant.

Repeated challenges of 10^{-3} M dopamine produced a series of contractions. The mean force developed during the first two contractions was 1·19 ± 1·00 g and 1·21 ± 0·84 g. The normalized efficacy of dopamine was 0·023 ± 0·019 and 0·023 ± 0·016 g (mg tissue)$^{-1}$, respectively. The amplitude of the following contractions decreased approximately 20% each time (Fig. 3, control). When the interval between two consecutive challenges was extended to 30 min, 1h or 3h, the tachyphylaxis persisted but took a slightly slower time course. Tachyphylaxis did not occur when the muscle strips were challenged repeatedly by either 10^{-6} M acetylcholine or 30 mM KCl (data not shown, 10^{-6} M is the EC50 of ACh).

The antagonist to dopaminergic receptors, haloperidol, significantly inhibited the dopamine-induced contraction at 10^{-5} and 10^{-4} M but did not alter the time course of tachyphylaxis (Fig. 2). The blockade was incomplete at 10^{-4} M and was not intensified by prolonged pretreatment.

Pretreating the muscle strips with the α-adrenoceptor antagonist, phentolamine 10^{-7} to 10^{-4} M, attenuated the contraction to dopamine (Fig. 3). Complete inhibition was reached at 10^{-4} M. The β-adrenoceptor antagonist, propranolol, significantly enhanced the contraction (10^{-8} to 10^{-6} M); however, tachyphylaxis persisted with propranolol-enhanced contractions to repeated challenges of dopamine (Fig. 4).

Discussion

The aim of our study was to determine the effect of exogenous dopamine on the canine tracheal smooth muscle. The results show that dopamine is an excitatory agent for this muscle (Fig. 1). Although repeated exposures to dopamine brought about desensitization of the muscle strips, the decreased response was not due to a decreased ability to contract. When the strips were no longer responsive to dopamine, they still contracted to ACh and KCl and histamine. Neither ACh nor KCl induced tachyphylaxis in these strips after repeated exposures.

The effective concentrations as well as the EC50 of dopamine established in this study agree well with those for dogs reported by Michoud et al (1985). Dopamine tachyphylaxis in guinea-pig trachea was reported by Advenier et al (1980) who also found that at 10^{-4} and 10^{-3} M it relaxed guinea-pig trachea previously contracted by ACh. Koga et al (1980) also found dopamine

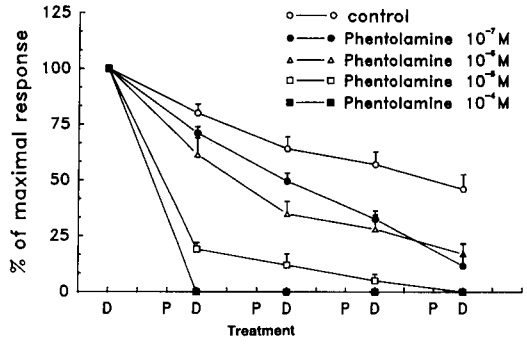

FIG. 3. Concentration-dependent inhibitory effect of an α-adrenergic antagonist, phentolamine (P), on contractions induced by repeated challenges of 10^{-3} M dopamine (D). Data are expressed as mean ± s.e. (n = 18 for control, n = 4 for each concentration of phentolamine). Phentolamine at all concentrations tested, 10^{-7}–10^{-4} M, significantly decreased the response to dopamine ($P < 0.05$).

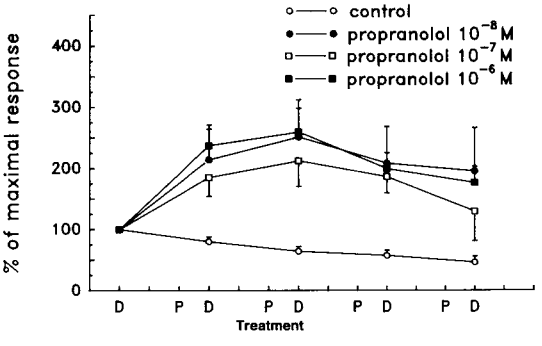

FIG. 4. Facilitatory effect of a β-adrenergic antagonist, propranolol (P), on contractions induced by repeated challenges of 10^{-3} M dopamine (D). Data are expressed as mean ± s.e. (n = 18 for control, n = 4 for each concentration of propranolol). Propranolol at all concentrations tested, 10^{-8}–10^{-6} M, increased the response to dopamine ($P < 0.05$). However, there is no statistical difference among the effects of propranolol at different concentrations.

(3×10^{-6} to 3×10^{-3} M) relaxed guinea-pig tracheal chain. Thus dopamine induced opposite responses in the tracheal preparations of dogs and guinea-pigs.

In the studies of guinea-pig isolated trachea, the effect of the dopaminergic antagonist, haloperidol, was controversial. At the highest concentration used (10^{-5} M), haloperidol inhibited 40% of the relaxation effect of dopamine (Advenier et al 1980). The study of Koga et al (1980) showed that haloperidol (10^{-5} and 3×10^{-5} M) did not affect the dopamine concentration-effect curve. Since the effect of dopamine was abolished by 10^{-6} M propranolol through competitive antagonism, both groups of authors suggested that the relaxant effect of dopamine is mainly β-adrenergic and the existence of dopaminergic receptors in guinea-pig trachea was excluded.

Our study has shown that haloperidol, 10^{-5} and 10^{-4} M, decreased the dopamine-induced contraction in canine tracheal smooth muscle (Fig. 2). Although the inhibitory effect of the α-adrenoceptor antagonist, phentolamine, began at a much lower concentration than that of haloperidol, complete inhibition was only possible at 10^{-4} M. Thus, in spite of the predominance of α-adrenergic activity in the contractile response to dopamine, the existence of dopaminergic receptors in canine tracheal smooth muscle cannot be ruled out. Nevertheless, the number of dopaminergic receptors may be limited, because, at the concentration of 10^{-4} M, haloperidol was unable to block completely the dopamine-induced contraction.

In canine tracheal smooth muscle, the dopamine-induced contraction was inhibited by an α-adrenoceptor and enhanced by β-adrenoceptor antagonist (Figs 3, 4). Thus dopamine, like noradrenaline and other α-adrenoceptor agonists, is capable of inducing simultaneous stimulation of α- and β-adrenoceptors. However, while blockade of β-adrenoceptors is required to bring out the contractile effect of noradrenaline, clonidine and phenylephrine on canine tracheal smooth muscle (Leff et al 1986), the contractile phase of dopamine response is not totally masked by the simultaneous β-adrenergic activation.

In conclusion, exogenous dopamine at high concentrations induced contraction in canine tracheal smooth muscle, but at lower concentrations did not cause relaxation. Repeated exposures to dopamine brought about desensitization of the muscle. While the actual site of action, neuronal or directly on smooth muscle, remains to be investigated, the contractile response to dopamine is predominantly due to the activity of α-adrenoceptors. The weakness of dopamine-induced contraction possibly results from an antagonism between α- and β-adrenergic effects. The dopamine tachyphylaxis may reflect a gradually decreased activation of the α-adrenergic mechanism in comparison with the β-adrenergic mechanism.

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