Blocking Action of Berberine on $\alpha_2$- and $\alpha_1$-adrenoceptors in Rat Vas Deferens and Anococcygeus Muscle

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Summary: Clonidine produced an inhibition of the electrical stimulation-evoked contraction of the prostatic portion of the rat vas deferens. Berberine and yohimbine suppressed markedly the effect of clonidine. However, washing away the clonidine by Krebs' solution produced a slow recovery of the responses.

Both berberine and yohimbine shifted clonidine cumulative dose-response curve rightward parallelly without decreasing the maximal response in rat vas deferens. The pA$_2$ values of berberine and yohimbine were 5.3 and 7.8 respectively.

The contractile response to phenylephrine was competitively inhibited by berberine and prazosin in anococcygeus muscle from the same rat. The pA$_2$ values of berberine and prazosin were 6.4 and 8.5 respectively.

The results indicate that berberine possesses competitive $\alpha_1$ and $\alpha_2$ blocking action like prazosin and yohimbine, and its selective ratio ($\alpha_1/\alpha_2$) was 8. As compared with these 2 drugs, the selectivity of berberine was much poorer, pD$_2$ values were 8.8 for clonidine and 5.6 for phenylephrine.

Key words: berberine, prazosin, yohimbine, phenylephrine, clonidine, anococcygeus muscle, vas deferens, drug dose-response relationship

In our previous communication we demonstrated that the contractile response to phenylephrine was competitively inhibited by berberine in rat anococcygeus muscle. In the present study the effects of berberine on the vas deferens and anococcygeus muscle preparation from the same rat were investigated, so as to evaluate further blocking action of berberine on $\alpha_1$- and $\alpha_2$-adrenoceptors.

MATERIALS AND METHODS

Male Wistar rats ($256 \pm 10$ g) were killed by a blow on the head. The vas deferens and anococcygeus muscle were rapidly dissected out, then suspended in an organ bath containing Krebs'
solution and bubbled with 95% O₂ + 5% CO₂ (pH=7.4). The specimens so obtained were maintained at 32°C in experiments with the vas deferens and at 37°C in those with the anococcygeus muscle. The tension development of isometric contractions in the isolated tissues was recorded by means of a mechano-electric transducer coupled to recorders.

1. α₂-adrenoceptor antagonist potency — rat vas deferens

The prostatic portion of the vas deferens was cleared of connective tissue and suspended under an initial tension of 1 g in a 10 ml organ bath.

An equilibration period of 1 h (tissue was washed 8–10 times with Krebs' solution for 1 h) was allowed before commencement of each experiment. 15 min before experiment the tissue was bathed in Krebs' solution containing hydrocortisone (40 μM), cocaine (30 nM) and prazosin (5 nM). The prostatic portion of the vas deferens was stimulated by square wave pulse of 3 ms duration at a frequency of 0.5 Hz using maximal voltage generated by stimulators about 9–10 V.

Agonist was added cumulatively to obtain dose-response curve for the inhibition of contractile response as control and then repeated in the presence of antagonist.

Antagonist potency was expressed as a pA₂ value which was calculated according to the Van Rossum method[3].

2. α₁-adrenoceptor antagonist potency — rat anococcygeus muscle

The two anococcygeus muscles[4] obtained from the same animals as used for the vas deferens experiments were suspended in a 10 ml organ bath using an initial tension of 0.5 g. The tissue was bathed in Krebs' solution containing hydrocortisone (40 μM), cocaine (30 nM) and propranolol (100 nM). Cumulative phenylephrine concentration contractile response curves were constructed in the absence and presence of the test antagonist. Postjunctional α₁-adrenoceptor antagonist potency was expressed as a pA₂ value (Van Rossum method).

3. Effect of berberine and prazosin on the contractile response to phenylephrine in epididymal portion of rat vas deferens (α₁ test)

At first agonist-phenylephrine was added to the bath and contractile response was evoked. When response reached its maximum, the tissue was washed several times with Krebs' solution until the vas deferens contractile tension returned to its premedicational level. Then the tissue was incubated with berberine or prazosin for 5 min respectively. At the end of incubation addition of phenylephrine and contractile response of vas deferens to phenylephrine was reconstructed.

4. Effect of clonidine, berberine and yohimbine on the contractile response to electrical stimulation in prostatic portion of vas deferens (α₂-adrenoceptor, time-effect relationship)

The tissue was stimulated with electrical stimulation, 15 min after equilibrium 10 nM of clonidine were added. Clonidine-evoked contractile change of vas deferens was continuously recorded at 1, 2, 4, 6, 8, 10 min. When clonidine-suppressed contractile response of vas deferens reached its nadir, cumulative doses of antagonist were added until contractile response reached its maximum.

RESULTS

1. Influence of berberine and yohimbine on effects of clonidine in prostatic portion of rat vas deferens

Cumulative dose of clonidine (0.1 nM—30 nM) produced an inhibition of electrical stimulation-evoked contractile response of the prostatic portion of vas deferens until it reached maximum, pD₂ value of clonidine was 8.8.

10 μM of berberine and 1 μM of yohimbine shifted clonidine dose-response curve to the right in parallel fa-