

# Effects of Muscone, Androsterone and Other Musk Components on Isoproterenol-induced Contractile Tension in Kitten and Guinea Pig Papillary Muscles

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(Received June 12, 1984)*

## Abstract

Musk and its known components (muscone, androsterone and glycerol) were studied for their effects on isoproterenol-induced contractile tension in kitten and guinea pig papillary muscle preparations. Musk potentiated the response to isoproterenol in both preparations, while androsterone did it only in kitten muscles, and the other known components never showed such an effect. It remains to obtain a trace of unknown substance (s) with an isoproterenol potentiating activity from a large quantity of musk.

**Keywords** Musk, Muscone, Androsterone, Glycerol, Isoproterenol, Potentiation, Papillary muscle

**Abbreviations** ED<sub>50</sub>; median effective dose, IR; infrared, NMR; nuclear magnetic resonance

## Introduction

An animal product, musk, is an important crude drug in Japan, and used as a cardiotoxic in traditional medicine.<sup>1)</sup> Musk is a soft, unctuous, brown and sometimes grainy substance, which is a dried secretion from the preputial follicle of musk deer. The substance at a concentration of 0.1mg/ml showed a positive inotropic effect in isolated, perfused frog heart preparations, but no increase in developed tension in kitten and rabbit papillary muscles.<sup>2)</sup> A water-soluble extract of musk, however, enhanced an effect of isoproterenol on contractile tension in kitten papillary muscles.<sup>3,4)</sup> We searched for the active components from musk, and obtained a partially purified substance (W<sub>5-a</sub>).<sup>5)</sup>

The purpose of this study is to perform further purification of W<sub>5-a</sub>, and to examine dose

-response relationships of known components from musk (muscone<sup>6)</sup> and androsterone as a representative of androstan derivatives<sup>7)</sup> concerning an isoproterenol potentiating activity in kitten and guinea pig papillary muscles, compared with those of musk and/or W<sub>5-a</sub>.

## Materials and Methods

**Animals**: Kittens of both sexes weighing 0.6-1.5kg and male Hartley guinea pigs weighing 600-700g were sacrificed to obtain papillary muscle (s) from the right ventricle.

**Tests on Isoproterenol Potentiating Activity**: A kitten papillary muscle preparation was suspended in an organ bath containing 2.5ml of a modified Lock's solution at 37°C, one end of the muscle being tied to a strain gauge transducer. The muscle preparation was driven by a given electric stimulation (10V, 1 msec and 0.4Hz), and the developed tension was recorded on an ink-writing oscillo-

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graph. In the case of guinea pig papillary muscles, the bath temperature of 30°C and the duration of electric stimulation of 5 msec were used instead. Isoproterenol was directly added to the bath, and the responses of the muscles to cumulative doses of isoproterenol (0.001-1 $\mu$ M for kitten muscles, 0.001-0.1 $\mu$ M for guinea pig muscles) were recorded. Cumulative dose-response curves for isoproterenol in the presence and absence of a tested compound were produced in turn, and ED<sub>50</sub> values of paired curves were estimated to calculate a potentiation ratio previously reported as an index of isoproterenol potentiating activity. The duration of pretreatment with musk or its component was 10 min in both muscle preparations to obtain the maximal effect.

*Preparation of Musk Fractions:* Purification of the active component from musk was performed using Nepali products via Hong Kong. Musk was first extracted with ether in Soxley apparatus for 20 hours to remove ether-soluble components, e.g. muscone, wax and cholesterol. The extraction was repeated 3 times, and the

residue was next extracted with ethanol at 40°C for 9 hours as a total. The ethanol-soluble extract was concentrated, dried, and then shaken in distilled water. The water-soluble substance (W<sub>5</sub>) was further fractionated by applying to an alumina column followed by eluting with ethanol. The eluate (W<sub>5-a</sub>) showed one spot detectable by heating with 10% H<sub>2</sub>SO<sub>4</sub> in thin-layer chromatography (TLC) on silica gel. The spot had a different location from those seen in the case of muscone and androstans.

*Isolation of Glycerol from W<sub>5-a</sub>:* A transparent, colorless and viscous substance was isolated from W<sub>5-a</sub> by preparative TLC on Merck Kieselgel 60 PF<sub>254</sub> with BuOH-AcOH-H<sub>2</sub>O (4 : 1 : 2). The musk component was identified with glycerol in comparison between the IR absorptions and the NMR spectra of the component and the authentic substance.

*Other Compounds:* Muscone was obtained from Kokando Co., Ltd. (Toyama). Androsterone and glycerol were obtained from Nakarai Chemicals, Ltd. (Kyoto).

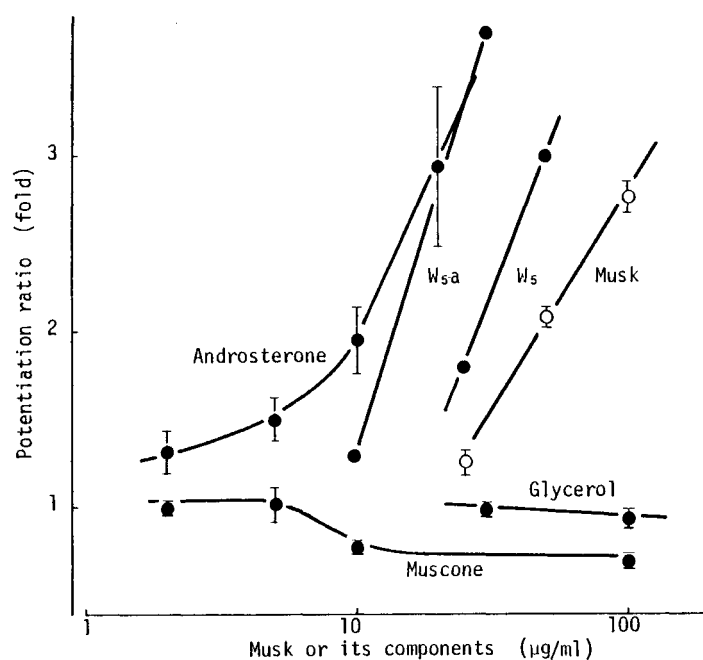


Fig. 1 Effects of Musk and its Components of Isoproterenol-induced Contractile Tension in Kitten Papillary Muscles. Each point with vertical bars represents mean  $\pm$ S.E. of 5-10 muscles. Each point without vertical bars, seen in the case of W<sub>5</sub> and W<sub>5-a</sub>, represents the mean of two muscles.

## Results

### 1. Isoproterenol Potentiation in Kitten Papillary Muscles

Musk and its components were studied for their actions on inotropic functions in kitten papillary muscles. Each compound tested alone showed no effect on the basic contractile tension in the muscles at any concentrations used. As shown in Fig. 1, however, isoproterenol-induced contractile tension was potentiated with crude musk,  $W_5$ ,  $W_{5-a}$ , and androsterone in a dose-dependent manner. Androsterone was about a 5-fold more potent potentiator than crude musk. Muscone, a fragrant component, never potentiated, but inhibited the response to isoproterenol. Glycerol, which was found to be a major composition of  $W_{5-a}$ , had almost no effect on isoproterenol-induced contractile tension.

### 2. Isoproterenol Potentiation in Guinea Pig Papillary Muscles

Using papillary muscles obtained from guinea pigs, inotropic and isoproterenol-potentiating activities of musk and its components were investigated. All the compounds tested

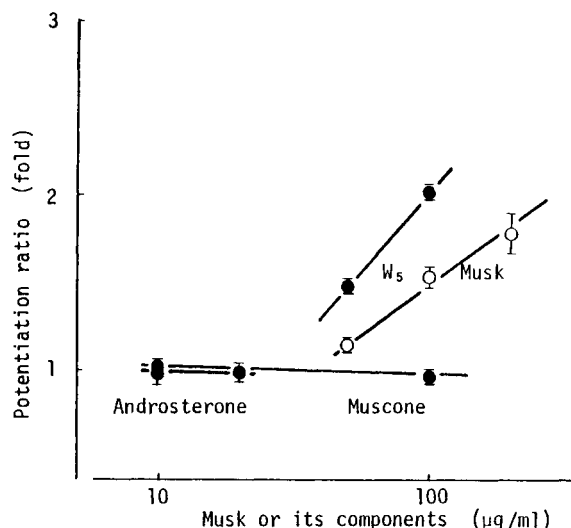


Fig. 2 Effect of Musk and its Components of Isoproterenol-induced Contractile Tention in Guinea Pig Papillary Muscles. Each point with vertical bars represents mean  $\pm$  S.E. of 6 guinea pigs.

showed no direct effect on the basic contractile tension in the muscles like the case seen in kitten papillary muscles. Fig. 2 shows that musk and its fraction  $W_5$  potentiated isoproterenol-induced contractile tension in guinea pigs like kittens, although the magnitude of potentiation was about a half. On the other hand, androsterone never showed isoproterenol potentiation in guinea pig papillary muscles, and muscone had also no effect.

## Discussion

In the course of our search for substance (s) with an isoproterenol-potentiating activity from musk fraction  $W_{5-a}$ , we isolated glycerol, which was a biologically inactive substance. When the ratio of glycerol to  $W_{5-a}$  was found to be at least 95%, we imagined that our target component would be a trace of substance present in musk.

In this situation, we attempted to determine whether known components from musk would enhance isoproterenol-induced contractile tension more potently than  $W_5$  or  $W_{5-a}$  did. Muscone, a fragrant component from musk, never showed any potentiating effect. On the other hand, androsterone possessed an isoproterenol potentiating activity in kitten papillary muscles. In guinea pig muscles, however, such an effect was not shown at all, being different from the case of  $W_5$  or  $W_{5-a}$ . A key difference in pharmacological activities between our target component and androsterone was found in addition to a difference in the solubility in organic solvents between them.

It remains to obtain a trace of the substance which potentiates the response to isoproterenol in both kitten and guinea pig papillary muscles. About 60 mg of  $W_{5-a}$  was obtained from 20g of musk.<sup>5)</sup> We imagine that our target component will be 1% or less of  $W_{5-a}$ . It is a big problem whether we could obtain a large quantity of musk.

## Acknowledgement

We acknowledge Dr. Matao Kanaoka, Research Institute for WAKAN-YAKU, Toyama Medical

and Pharmaceutical University, for his kind advice about IR and NMR studies, and Miss Tomoko Yamazaki for her technical assistance. This work was supported in part by grants-in-aid from the Foundation for the Promotion of Research on Medicinal Resources (Tokyo) and from the Ministry of Health & Welfare in Japan.

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