INFLUENCE OF FENTANYL ON THE CHRONOTROPIC RESPONSE OF ISOLATED RABBIT ATRIA TO CHOLINERGIC AND ADRENERGIC STIMULATION

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Abstract—Effects of fentanyl and morphine on chronotropic responses to transmural stimulation applied to the S-A node were studied in isolated rabbit atria. The stimulation caused a frequency-dependent negative chronotropism followed by positive chronotropism, the former abolished by atropine and the latter suppressed by propranolol. The former response at 5 and 20/sec was attenuated in a dose-dependent manner by fentanyl and morphine. Potency ratio of fentanyl to morphine was approx. 50–100. The inhibitory effect of fentanyl and morphine on the response to stimulation of cholinergic nerves was partially prevented or reversed by both naloxone and levallophan, and also partially reversed by repeated washing of the preparations. Antagonism by naloxone was appreciably greater than that of levallophan. The negative chronotropic response to transmural stimulation was reduced by levallophan but not by naloxone. Dose-chronotropic response curves of acetylcholine were unaffected by treatment with fentanyl and morphine. High concentrations of fentanyl and morphine attenuated the response to stimulation of adrenergic nerves, while dose-chronotropic response curves of noradrenaline were not influenced by these narcotics. Naloxone was ineffective in preventing or reversing the effect of fentanyl and morphine. It is therefore concluded that fentanyl and morphine activate opiate receptors located in cholinergic nerve terminals innervating the S-A node, thereby interfering with the release of acetylcholine. Further, it appears that high concentrations of fentanyl and morphine interfere with the release of noradrenaline from postganglionic adrenergic nerve terminals in rabbit atria; however, this action is not related to opiate receptors.

It has been demonstrated that morphine and its analogues depress the response to electrical stimulation of cholinergic nerves innervating the guinea pig ileum (1, 2, 3) and the rabbit sinoatrial (S-A) node (4), possibly by interference with the release of acetylcholine. The inhibition seen in the ileum appears to be related the action of the analgesics on opiate receptors, since naloxone antagonizes the inhibition (5, 6). On the other hand, morphine inhibits contractions elicited by adrenergic neural stimulation of the cat nictitating membrane in situ and in vitro (7, 8, 9) and of isolated mouse vas deferens (10).

Fentanyl, a potent morphine-like analgesic narcotic (11, 12, 13), is widely used for neuroleptanesthesia. However, little information is available concerning the effect of this narcotic on the peripheral autonomic nervous system. The present study describes inhibition by fentanyl of the chronotropic response to electrical stimulation of postganglionic cholinergic and adrenergic nerves innervating the rabbit S-A node, as compared with the action of morphine. Reversal of the inhibition by narcotic antagonists was also investigated.
MATERIALS AND METHODS

Ninety-five albino rabbits of both sexes, weighing 1.8 to 2.5 kg, were used. Under ether anesthesia, the animals were bled from both common carotid arteries. The heart was rapidly removed and the S-A node-right atrium preparation was prepared in oxygenated nutrient solutions. The specimen was fixed horizontally between hooks under a resting tension of 300 to 450 mg in a muscle bath of 60-ml capacity containing the nutrient solution. Hooks anchoring the right atrial appendage were connected to the lever arm of a force-displacement transducer (Nihonkohden Kogyo Co.). The bathing medium was maintained at 30±0.5°C and gassed with a mixture of 95% O₂ and 5% CO₂. The composition of the nutrient solution was as follows (mM); Na⁺, 162.1; K⁺, 5.4; Ca²⁺, 2.2; Cl⁻, 157.0; HCO₃⁻, 14.9; dextrose, 5.6. During the equilibration period, the bathing medium was replaced at intervals of 10 to 30 minutes with fresh media.

A monopolar silver electrode, 0.5 mm in diameter and insulated to the tip, was used for transmural electrical stimulation of intracardiac cholinergic and adrenergic nerves innervating the S-A node (14). The transmural stimulation was the local application of square pulses, 0.1 msec in duration and 5 to 10 mA in intensity (supramaximum for the stimulation of nerve terminals) applied at frequencies of 5, 20 and 100/sec for a period of 3 seconds. Electrical pulses were delivered from an electronic stimulator (Nihonkohden Kogyo Co.).

Isometric contractions of the right atrium were displayed on an ink writing oscillograph (Sanei Sokki Co.). The cycle length was measured under steady-state conditions and also when the maximum response was attained after transmural stimulation. Transmural stimulation was applied repeatedly until steady state responses were attained. The S-A nodal rate was calculated from the maximum cycle length between contractions when the negative chronotropic response to transmural stimulation was induced, and from mean values of 10 measurements of the cycle length when the positive chronotropic response was induced. The results were expressed as mean values ± S.E.M. Comparisons of results were made using Student’s t-test.

Drugs used were dl-noradrenaline hydrochloride, acetylcholine chloride, atropine sulfate, dl-propranolol hydrochloride, hexamethonium bromide, bretylium tosylate, tetrodotoxin, fentanyl citrate, morphine hydrochloride and levallorphan tartrate, all of which were applied directly to the muscle bath. Cumulative dose-response curves of acetylcholine and noradrenaline were obtained. Preparations were exposed for 30 minutes to fentanyl, morphine, naloxone or levallorphan before the transmural stimulation was applied or before dose-response relationships of acetylcholine and noradrenaline were obtained.

RESULTS

Modification by fentanyl and morphine of the negative chronotropic response to transmural electrical stimulation

Transmural electrical stimulation applied to the S-A node caused a frequency-dependent decrease in atrial rate followed by an increase in the rate as shown in Table 1. The negative
The chronotropic response to transmural stimulation was abolished by treatment with 10^{-6} M atropine and 10^{-7} M tetrodotoxin but was unaffected by 10^{-8} M hexamethonium. The positive chronotropic response was abolished by 10^{-5} M bretylium, 10^{-6} M propranolol or 10^{-7} M tetrodotoxin.

The addition of fentanyl in concentrations of 10^{-6} M and 5 \times 10^{-6} M slowed the atrial rate: mean values of the rate in control and fentanyl (10^{-6} and 5 \times 10^{-6} M)-treated preparations were 97.8 \pm 3.5, 93.0 \pm 4.3 and 84.1 \pm 3.9 beats/min (N=11), respectively. Atropine (10^{-6} M) and naloxone (10^{-6} M) failed to reverse and to prevent the effect of fentanyl. Treatment with fentanyl in concentrations in a range between 2 \times 10^{-7} M and 5 \times 10^{-6} M produced a dose-related inhibition in the negative chronotropic response to transmural electrical stimulation: the inhibition in the response at a frequency of 5/sec was markedly

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**Table 1. Negative and positive chronotropic responses to transmural electrical stimulation**

| Control rate (beats/min) | % change induced by stimulation at 5/sec | % change induced by stimulation at 20/sec | % change induced by stimulation at 100/sec |
|--------------------------|----------------------------------------|----------------------------------------|----------------------------------------|
| 104.0 \pm 2.4            | -21.8 \pm 2.0                          | -67.8 \pm 1.8                          | -79.1 \pm 1.4                          |
| +12.3 \pm 1.7            | +22.7 \pm 1.7                          | +25.8 \pm 2.0                          |                                        |

Number of preparations used: 60. -, decrease in atrial rate; +, increase in rate.

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**Fig. 1.** Attenuation by fentanyl of the negative chronotropic response of atria to transmural stimulation in control (left figure) and propranolol (10^{-6} M)-treated preparations (right figure). Decreases in atrial rate induced by transmural stimulation at each frequency in control media were taken as 100 per cent. Mean values of the decrease in atrial rate induced by stimulation at frequencies of 5, 20 and 100/sec in control media were 13.1 \pm 5.3, 65.4 \pm 3.6 and 78.8 \pm 3.8 beats/min, respectively, in control preparations, and 17.5 \pm 5.7, 65.8 \pm 6.0 and 78.3 \pm 3.6 beats/min, respectively, in propranolol-treated preparations. F, fentanyl; after wash, after repeated washing of preparations soaked in 5 \times 10^{-5} M fentanyl. Figures in parentheses indicate the number of preparations used, and vertical bars represent standard errors of means, which are common to Figs. 1–7.
greater than that at the higher frequencies (Fig. 1, left). The maximum inhibition was attained after 30 min exposure to fentanyl. The inhibition induced by $5 \times 10^{-6}$ M fentanyl was partially reversed by replacement of fentanyl-containing fluids 3 times with control media. Similar inhibition by fentanyl was also observed in preparations treated with $10^{-5}$ M propranolol by which tachycardia following bradycardia induced by transmural stimulation was abolished (Fig. 1, right).

In preparations in which fentanyl ($5 \times 10^{-6}$ M)-induced inhibition in the negative chronotropic response to transmural stimulation had been established, naloxone at $10^{-6}$ M partially reversed the inhibition (significantly different at 5/sec, $p<0.001$ and at 20/sec, $p<0.01$) (Fig. 2, left). Further increase in the concentration to $10^{-5}$ M failed to produce an additional restoration. Levallorphan was also effective in restoring the response which had been attenuated by fentanyl (significantly different at 5/sec, $p<0.02$, and at 20/sec, $p<0.05$); however, the antagonistic effect of levallorphan was appreciably less than that with the same dose of naloxone (Fig. 2, right). Naloxone in concentrations up to $10^{-5}$ M failed to alter the negative chronotropic response to transmural stimulation in control media, while in contrast, levallorphan ($10^{-6}$ M) significantly attenuated the response to stimulation at 5, 20 and 100/sec by 16.5±3.5% ($p<0.001$), 13.3±4.1% ($p<0.01$) and 6.0±1.6 ($p<0.01$) (N=7), respectively.

The addition of morphine in concentrations of $10^{-5}$ M and $10^{-4}$ M failed to alter the atrial rate but at $10^{-3}$ M significantly slowed the rate to 81.6±4.7 beats/min from the pre-drug rate of 102.0±5.6 beats/min (N=13, $p<0.02$). The negative chronotropic effect of transmural stimulation was significantly attenuated by treatment with morphine ($10^{-5}$ and $10^{-4}$ M) (Fig. 3, left). Naloxone in a concentration of $10^{-6}$ M significantly reversed the attenuation of the response to stimulation at frequencies of 5/sec ($p<0.01$) and 20/sec

![Fig. 2. Reversal by naloxone (left figure) and levallorphan (right figure) of the fentanyl ($5 \times 10^{-6}$ M)-induced inhibition of the negative chronotropic response to transmural stimulation. Naloxone and levallorphan were added after the fentanyl-induced inhibition had been established. Mean values of the decrease in atrial rate induced by stimulation at frequencies of 5, 20 and 100/sec in control media were 12.1±2.2, 79.2±4.8 and 92.6±7.1 beats/min, respectively (for left figure), and 19.8±3.8, 65.2±4.1 and 77.9±4.0 beats/min, respectively (for right figure).]
The addition of acetylcholine in concentrations ranging from $2 \times 10^{-8}$ M to $10^{-3}$ M caused a dose-dependent decrease in the atrial rate. The dose-chronotropic response curve of acetylcholine was unaffected by treatment for 30 min with fentanyl ($10^{-6}$ and $5 \times 10^{-6}$ M) or morphine ($10^{-4}$ M) (Fig. 4).

The addition of acetylcholine in concentrations ranging from $2 \times 10^{-8}$ M to $10^{-8}$ M caused a dose-dependent decrease in the atrial rate. The dose-chronotropic response curve of acetylcholine was unaffected by treatment for 30 min with fentanyl ($10^{-6}$ and $5 \times 10^{-6}$ M) or morphine ($10^{-4}$ M) (Fig. 4).
FIG. 5. Modification by fentanyl of the positive chronotropic response of atria to transmural stimulation in control (left figure) and atropine (10^-6 M)-treated preparations (right figure). Increases in atrial rate induced by stimulation of adrenergic nerves in fentanyl-treated preparations were compared with those prior to the treatment with fentanyl, and % changes in rate increase are presented. Mean values of the increase in atrial rate induced by stimulation at frequencies of 5, 20 and 100/sec in control media were 14.2 ± 6.1, 22.2 ± 2.7 and 27.1 ± 4.7 beats/min, respectively, in control preparations, and 21.4 ± 7.4, 35.6 ± 7.7 and 44.4 ± 7.0 beats/min, respectively, in atropine-treated preparations.

FIG. 6. Modification by morphine of the positive chronotropic response to transmural stimulation. The ordinate represents % changes in the response. Mean values of the increase in rate at frequencies of 5, 20 and 100/sec in control media were 11.3 ± 2.1, 25.7 ± 4.4 and 25.5 ± 3.7 beats/min, respectively.
Modification by fentanyl and morphine of the positive chronotropic response to transmural stimulation and noradrenaline

Treatment with fentanyl at 2 \times 10^{-6} \text{ M} and 10^{-6} \text{ M} caused a slight increase in the positive chronotropic response to transmural stimulation at a frequency of 5/sec (Fig. 5, left). Increase in the concentration of fentanyl to 5 \times 10^{-6} \text{ M} significantly attenuated the positive chronotropic response to transmural stimulation at a frequency of 100/sec (p<0.01). In atropine-treated preparations, fentanyl at 10^{-6} \text{ M} and 5 \times 10^{-6} \text{ M} attenuated the positive chronotropic response to stimulation (Fig. 5, right). The fentanyl-induced attenuation was neither reversed nor prevented by naloxone (N=5) and levallorphan (N=10). The time required to restore the S-A nodal rate to the rate prior to transmural stimulation was prolonged in five out of six preparations treated with 5 \times 10^{-6} \text{ M} fentanyl; mean values of the time at frequencies of 5, 20 and 100/sec were 2.3 \pm 0.5, 4.2 \pm 0.9 and 4.7 \pm 1.2 min in control preparations and 3.3 \pm 0.5, 6.5 \pm 0.7 and 6.8 \pm 0.8 min in preparations treated with 5 \times 10^{-6} \text{ M} fentanyl, respectively (N=6).

Treatment with 10^{-4} \text{ M} morphine significantly attenuated the positive response to transmural stimulation at frequencies of 20/sec (p<0.05) and 100/sec (p<0.02) (Fig. 6).

The addition of noradrenaline in concentrations ranging from 5 \times 10^{-9} \text{ M} to 5 \times 10^{-5} \text{ M} caused a dose-dependent increase in the atrial rate. The dose-chronotropic response curve of noradrenaline was unaffected by treatment for 30 min with fentanyl (10^{-6} and 5 \times 10^{-6} \text{ M}) or morphine (10^{-4} \text{ M}) (Fig. 7).

DISCUSSION

Transmural electrical stimulation applied to S-A node under the experimental condition used in the present study produced a frequency-dependent bradycardia followed by a tachycardia. The initial bradycardia was abolished by atropine and tetrodotoxin and the
subsequent tachycardia by propranolol, bretylium and tetrodotoxin, suggesting that both responses are due to release of acetylcholine and noradrenaline, respectively, from autonomic nerves innervating the S-A node (14, 15). Fentanyl and morphine attenuated the negative chronotropic response to transmural neural stimulation in a dose-dependent manner but did not influence the response to acetylcholine. These findings are consistent with those seen with morphine in the rabbit atrium (4) and in the guinea pig ileum (1, 2, 16). Morphine does not interfere with the synthesis of acetylcholine (17) nor with the propagation of excitation along the nerves (18, 19). Furthermore, large doses of fentanyl failed to inhibit the neuromuscular transmission in anesthetized cats (12). Thus, it may be concluded that these narcotic analgesics inhibit the release of acetylcholine from autonomic cholinergic nerve terminals. The potency ratio of the inhibition of the response to the stimulation at 5/sec by fentanyl and morphine was approximately 50-100:1. Similar ratio of analgesic potency of fentanyl and morphine has been demonstrated in humans (20, 21).

Inhibition of the negative chronotropic response to transmural stimulation by fentanyl and morphine was partially reversed or prevented by both naloxone and levallorphan. Antagonism by naloxone was appreciably greater than that of levallorphan. The negative chronotropic response to transmural stimulation was not reduced by naloxone but by levallorphan. It has been demonstrated that levallorphan stimulates opiate receptors on the one hand and also antagonizes the stimulating effect of morphine on the opiate receptors on the other (5, 16, 22, 23). These findings strongly suggest that fentanyl and morphine activate opiate receptors located in the cholinergic nerve terminals innervating the S-A nodes, thereby interfering with the release of acetylcholine.

In the present study with isolated atria, fentanyl and morphine elicited a greater attenuation of the response to stimulation of cholinergic nerves at low frequencies than at high frequencies. Furthermore, naloxone and levallorphan antagonized the inhibitory effect of fentanyl and morphine on the response to stimulation at low frequencies to a greater extent. It has been postulated that two mechanisms in the release of acetylcholine from the electrically-stimulated ileum may exist; one at low frequencies of stimulation is morphine sensitive, and the other at high frequencies of stimulation is morphine resistant (24).

It has been demonstrated that intravenous injections of fentanyl slow the heart rate of intact animals and humans, and such rate slowing is cholinergic and of central origin since the response is abolished by atropine and naloxone (12, 25). In contrast, bradycardia induced by high concentrations of fentanyl in isolated rabbit atria was not influenced by pre-treatment with atropine and naloxone, suggesting that cholinergic and opiate receptors are not involved.

Fentanyl in low concentrations tended to potentiate the positive chronotropic response to transmural stimulation. The duration of the response was also prolonged. Fentanyl as well as morphine have been reported to interfere with the re-uptake of noradrenaline by adrenergic nerve terminals (26). Drugs that inhibit the neuronal uptake of noradrenaline such as cocaine and desipramine reportedly potentiate the positive chronotropic effect of sympathetic nerve stimulation and prolong the effect (27). High concentrations of fentanyl
and morphine reduced the positive chronotropic response to transmural stimulation but not the response to noradrenaline. Naloxone failed to reverse or prevent the inhibitory effect of the narcotics. It appears that fentanyl and morphine in high concentrations interfere with the release of noradrenaline from adrenergic nerve terminals; however, this action is not related to opiate receptors.

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