Oxybutynin Influence on Autonomic Measures in Dogs

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Abstract □ In the conscious dog, the most apparent oxybutynin effect was a dose-related tachycardia. Associated with this heart rate increase were a very slight, sometimes significant, elevation in diastolic pressure and an insignificant increase in systolic pressure. Under pentobarbital anesthesia, the systolic/diastolic arterial pressure oxybutynin responses were reversed and showed a dose-related systolic and diastolic hypotension. However, the tachycardic response to oxybutynin still appeared. The arterial pressure and heart rate responses produced by the autonomic agents were altered by the oxybutynin treatment in a pattern indicative of an anticholinergic mechanism of action. Differences in many response profiles were observed with either conscious or anesthetized dogs, but the statistically significant inhibition of the acetylcholine-induced systolic and diastolic arterial pressure and bradycardic responses were constant in both conditions. Oxybutynin is an anticholinergic agent with mild to moderate cardiovascular activity.

Keyphrases □ Oxybutynin—effect on autonomic functions in conscious and phenobarbital-anesthetized dogs □ Autonomic functions—effect of oxybutynin, conscious and phenobarbital-anesthetized dogs □ Phenobarbital—effect on oxybutynin response, autonomic functions, dogs

Previous studies indicated that the tertiary amine oxybutynin chloride (4-diethylamino-2-butynyl α-phenylcyclohexaneglycolate hydrochloride) had a weakly anticholinergic mechanism of action. In an anesthetized dog, a cumulative intravenous dose of 750 μg/kg (last dose of 400 μg/kg) completely blocked the hypotensive acetylcholine-induced response and partially (60%) inhibited the hypotensive response to vagal stimulation. Atropine, 10 μg/kg, produced a 66% reduction of the vagal blood pressure response and a 30% inhibition of the acetylcholine depressor response.

Table I—Effects of Graded Intravenous Oxybutynin Doses on Mean Basal Level and Mean Response Amplitude in Six Conscious Beagle Dogs

Treatment	Systolic, mm Hg	Diastolic, mm Hg	Heart Rate, beats/min	Respiration Rate, breaths/min
Basal level	133 (8.9)[a]	87 (6.2)	107 (5.3)	42 (5.5)
Oxybutynin				
0.1 mg/kg	+5 (3.4)	+1 (0.8)	+1 (2.6)	+8 (4.3)
0.3 mg/kg	+3 (4.3)	+2[b] (0.7)	+21[b] (5.5)	+6 (5.9)
1.0 mg/kg	+9 (5.9)	+6[b] (2.4)	+35[b] (11.8)	+2 (0.9)
3.0 mg/kg	−7 (8.8)	+3 (6.7)	+52[b] (12.5)	+7 (3.5)

[a] Standard errors are given in parentheses. [b] $p \leq 0.05$.

Table III—Effects of Graded Intravenous Oxybutynin Doses on Mean Basal Level and Mean Response Amplitude in Six Anesthetized Beagle Dogs

Treatment	Systolic, mm Hg	Diastolic, mm Hg	Heart Rate, beats/min	Respiration Rate, breaths/min
Basal level	125 (9.5)[a]	89 (9.3)	140 (8.2)	22 (5.1)
Oxybutynin				
0.1 mg/kg	0	+2 (1.1)	+4 (2.4)	+1 (0.7)
0.3 mg/kg	0	0	+1 (2.2)	+1 (0.3)
1.0 mg/kg	−8 (4.6)	−5 (3.2)	+12[b] (2.0)	+1 (0.6)
3.0 mg/kg	−30[b] (6.2)	−26[b] (5.8)	+25[b] (5.7)	+6 (2.9)
10.0 mg/kg	−31[b] (2.4)	−37[b] (3.7)	+1 (3.5)	+17[b] (6.3)

[a] Standard errors are given in parentheses. [b] $p \leq 0.05$.

The physiological state of the experimental preparation can influence the quality and amplitude of a response profoundly. In the anesthetized dog, the alkaloid ibogaine produced a marked hypotension, which was not evident in the conscious dog (1). In anesthetized dogs, the alkaloid yohimbine lowered arterial pressure due to a peripheral α-adrenergic blocking effect (2). In these same animals, while conscious, an equivalent intravenous yohimbine dose produced a hypertension and tachycardia; both responses endured for 30 min or longer. The response from the conscious dog was an expression of the yohimbine central sympathetic nervous system stimulating effect, which was not evoked in the anesthetized (barbiturate) animal.

A recent study in chronically prepared dogs investigated anesthesia influence on cardiovascular responses to cryptenamine, an alkaloid from *Veratrum veride* (3). In

Table II—Effects of a Cumulative Oxybutynin Dose of 4.4 mg/kg iv on Autonomic Response in Six Conscious Beagle Dogs

Treatment	Systolic, mm Hg	Diastolic, mm Hg	Heart Rate, beats/min	Respiration Rate, breaths/min
		Control Response		
Bilateral carotid occlusion	+92 (73)[a]	+45 (54.2)	+20 (12)	+2 (1)
Tyramine	+58 (18.8)	+22 (4.6)	+18 (3.6)	+1 (0.8)
Acetyl choline	−21 (6.1)	−17 (5.3)	+20 (11.3)	+4 (1.2)
Epinephrine	+33 (4.9)	+21 (3.5)	+11 (5.7)	+4 (5.4)
Histamine	−26 (2.4)	−23 (3.1)	+48 (10.7)	+1 (1)
Norepinephrine	+34 (4.7)	+18 (7)	−2 (1.9)	+5 (2.3)
Isoproterenol	−29 (8.6)	−30 (8.8)	+78 (13.8)	+23 (7.4)
5-Hydroxytryptamine	+29 (9.2)	+13 (5.1)	+12 (10.5)	−2 (4.3)
		After Oxybutynin		
Bilateral carotid occlusion	+48 (17.4)	+33 (8.3)	−4 (4.9)	+1 (1.8)
Tyramine	+75 (21.3)[b]	+42 (9.8)[b]	+3 (1.3)[b]	+11 (6.7)
Acetylcholine	−11 (4.3)[b]	−3 (4.9)[b]	+4 (7.6)[b]	−10 (9.1)
Epinephrine	+42 (11.8)	+27 (5.2)[b]	−1 (4)[b]	+13 (10.2)
Histamine	−30 (3.5)	−35 (6.8)	+83 (19.4)[b]	−3 (4.4)
Norepinephrine	+32 (6.3)	+14 (5.4)	−28 (1)[b]	+4 (2.4)
Isoproterenol	−36 (8.9)	−55 (8.4)[b]	+54 (11.1)	+12 (9.2)
5-Hydroxytryptamine	+17 (5.9)	+9 (4.3)	−4 (4.3)	+8 (10)

[a] Standard errors are given in parentheses. [b] $p \leq 0.05$.

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Table IV—Effects of a Cumulative Oxybutynin Dose of 14.4 mg/kg iv on Autonomic Response in Six Anesthetized Beagle Dogs

Treatment	Systolic, mm Hg	Diastolic, mm Hg	Heart Rate, beats/min	Respiration Rate, breaths/min
		Control Response		
Bilateral carotid occlusion	+11 (6.7)[a]	+17 (1.7)	+35 (35)	+3 (4.1)
Tyramine	+122 (30.5)	+49 (20.5)	+18 (17)	−3 (1.8)
Acetylcholine	−26 (2.4)	−27 (3.1)	+21 (5.3)	+2 (1.5)
Epinephrine	+33 (7)	+22 (8)	−1 (3.9)	+4 (1.6)
Histamine	−24 (3.3)	−28 (6.7)	+12 (4.8)	+2 (1.3)
Norepinephrine	+39 (5.2)	+27 (5.7)	−22 (4.5)	0
Isoproterenol	−32 (9.2)	−45 (5.2)	+101 (11.1)	+11 (3.8)
5-Hydroxytryptamine	+5 (4.5)	+1 (2.7)	+6 (5.8)	+7 (4.1)
		After Oxybutynin		
Bilateral carotid occlusion	+15 (3)	+27 (12.5)	+48 (17)	+2 (0.5)
Tyramine	+106 (30.3)	+61 (23.3)	+53 (15.6)	+7 (7.1)
Acetylcholine	+1 (0.8)[b]	+1 (1.5)[b]	−3 (2.9)[b]	+2 (0.9)
Epinephrine	+76 (13.8)[b]	+39 (14.4)[b]	+22 (12.9)[b]	+24 (21.7)
Histamine	−30 (3.9)	−27 (7.9)	+10 (1.4)	+6 (2.8)
Norepinephrine	+92 (11.8)[b]	+47 (7.2)[b]	+21 (10.5)[b]	+2 (0.6)[b]
Isoproterenol	−34 (6.9)	−59 (3.3)[b]	+82 (8.3)	+8 (4)
5-Hydroxytryptamine	0	−5 (5.4)	+1 (1.8)	+10 (3.2)

[a] Standard errors are given in parentheses. [b] $p \leq 0.05$.

32 paired conscious and anesthetized dogs, pentobarbital anesthesia significantly lowered basal systolic/diastolic arterial pressures, raised basal heart rate, and altered responses to autonomic treatments. Pentobarbital anesthesia also significantly increased the cryptenamine dose required for inhibition of the hypotensive and/or bilateral carotid occlusion pressor response. The conscious dog systolic depressor response to cryptenamine is the most sensitive and accurate bioassay for cryptenamine potency evaluation.

Earlier studies on oxybutynin were performed only in anesthetized animals. This paper reports its effects on autonomic functions and responses to autonomimetic treatments in conscious and anesthetized matched paired dogs.

EXPERIMENTAL

Six male and female beagle dogs were surgically prepared with externalized bilateral carotid artery loops (4). Each animal was its own control and was utilized both conscious and anesthetized (~35 mg of pentobarbital sodium/kg iv). This preparation permits direct systolic and diastolic arterial pressure measurement, induction of a reflex pressure response to bilateral carotid occlusion, and measurement of other autonomic functions such as ECG, heart rate, and respiration on conscious, slightly restrained animals (5).

Direct systolic and diastolic arterial pressure (lateral) was measured from an externalized carotid artery cannula[1]. The cannula was connected to a pressure transducer[2], which supplied input into a recorder coupler[3]. Cannula patency was maintained by slow retrograde saline infusion through the pressure transducer. The infusion rate did not alter the arterial pressure measurements.

Heart rate was measured by the conversion of the R-R wave interval by a cardiotachometer coupler[4] from a bipolar electrocardiograph input obtained through biopotential electrodes positioned and affixed to the skin over the sternum. A homemade sodium chloride electrode paste (0.154 mole/kg)[5] was used to facilitate conduction.

Respiration rate and amplitude were measured indirectly through a pneumograph belt positioned around the thoracico-abdominal junction and connected to a pressure transducer[2], which was connected to a coupler[3].

Recording was on a multichannel recorder[6] at a paper speed of 5 mm/sec.

[1] Medicon AR-3218.
[2] Statham model P-23AA.
[3] Beckman strain-gauge coupler 9872.
[4] Beckman cardiotachometer coupler 9857.
[5] In Unibase.
[6] Beckman type R dynograph.

Table V—Difference in Mean Basal Level and Mean Amplitude of Cardiovascular Response to Graded Oxybutynin Doses in Six Beagle Dogs Examined in the Conscious and Anesthetized States

Treatment	Systolic, mm Hg	Diastolic, mm Hg	Heart Rate, beats/min	Respiration Rate, breaths/min
Basal level	+8	−2	−33[a]	+20[a]
Oxybutynin				
0.1 mg/kg	+5	−1	−4	+7
0.3 mg/kg	+3	+2	+20[a]	+6
1.0 mg/kg	+16[a]	+11[a]	+23[a]	+1
3.0 mg/kg	+23[a]	+29[a]	+27[a]	+1

[a] $p \leq 0.05$.

To control for order effect, the six animals were treated in a random order in both the conscious and anesthetized states.

All dogs had an indwelling venocatheter (PE-50) in the branchial-cephalic or saphenous vein for drug solution administration. They were anesthetized with pentobarbital sodium, 30–35 mg/kg. Care was taken to position the pressure transducer at the appropriate level (heart horizontal) for each dog, particularly when anesthesia was employed.

Fresh aqueous oxybutynin solutions were prepared prior to use, and the concentrations were adjusted so that equivolumes were administered at each dose level. The oxybutynin doses were 0.1, 0.3, 1.0, 3.0, and 10.0[7] mg/kg iv in equivolumes of 0.1 ml/kg. Systolic and diastolic arterial pressures, heart rate, ECG, and respiration rate were measured.

After a 10–15-min equilibration, the animal received an autonomic treatment series. The autonomic agonists and their dose levels (salt weight) were: bilateral carotid occlusion, 30 sec; tyramine hydrochloride, 0.20 mg/kg; acetylcholine chloride, 0.6 μg/kg; epinephrine hydrochloride, 2 μg/kg; histamine phosphate, 2 μg/kg; norepinephrine bitartrate, 2 μg/kg; isoproterenol hydrochloride, 2 μg/kg; and serotonin diphosphate, 10 μg/kg. The autonomic treatments were repeated after the highest oxybutynin dose. The direction and amplitude of the autonomic responses were described previously (6).

The data were analyzed for statistical significance by the matched-pair t test (7).

RESULTS AND DISCUSSION

The data are summarized in Tables I–IV.

Oxybutynin effects in the conscious dog are reviewed in Tables I and II. At a mean basal systolic arterial pressure level of 133 mm Hg, oxybutynin doses of 0.1, 0.3, 1.0, and 3.0 mg/kg iv produced statistically insignificant changes in systolic pressure (Table I). The mean basal diastolic pressure of 87 mm Hg was significantly elevated following treatment with 0.3 and 1.0 mg of oxybutynin/kg. Diastolic pressure increased insignificantly (3 mm Hg) after the 3.0-mg/kg dose. These changes in diastolic

[7] Given only if an absent or a weak response resulted from lower dose.

pressure were statistically significant but physiologically inconsequential.

Oxybutynin produced apparently dose-related tachycardia. A significant peak tachycardic response (21 beats/min) appeared after the 0.3-mg/kg dose, and a significant peak (52 beats/min) occurred after the 3.0-mg/kg iv dose. Although there was a consistent tachypnea after each oxybutynin dose, none of the respiratory changes was significant.

The effects of the cumulative 4.4-mg/kg iv oxybutynin dose on the autonomic responses in conscious dogs are summarized in Table II. Oxybutynin produced several significant autonomic responses. The tyramine-induced pressor response was significantly enhanced. The tyramine-evoked tachycardic response was significantly depressed to +3.2 beats/min. The acetylcholine-induced depressor and associated tachycardic responses were significantly inhibited. The epinephrine pressor response amplitude was significantly enhanced by oxybutynin treatment, although the associated heart rate response was inhibited by from +11 to −1 beats/min. Other significant changes included enhanced histamine tachycardia, increased norepinephrine brachycardia, and increased diastolic depressor response to isoproterenol.

When oxybutynin was administered to anesthetized dogs, the systolic and diastolic pressure responses were opposite to those observed in the conscious state (Table III). Oxybutynin (1.0, 3.0, and 10.0 mg/kg iv) produced systolic/diastolic depressor responses of −8/−5, −30/−26, and −31/−37 mm Hg, respectively. The associated significant tachycardic responses occurred only after the 1.0- and 3.0-mg/kg doses. The 10.0-mg/kg oxybutynin dose produced profound and significant depressor responses but insignificantly increased heart rate. Perhaps at this dose in the anesthetized dog the heart rate response, which appears to be compensatory to the systolic/diastolic depression, is pharmacologically antagonized by oxybutynin. The possibility of a toxicologic response to the 10.0-mg/kg dose is supported by the appearance of a marked and statistically significant tachypnea (+17 breaths/min).

Table IV describes the effects of a total cumulative 14-mg/kg iv oxybutynin dose of the autonomic treatment responses of anesthetized dogs. The acetylcholine-induced arterial pressure depressor response was inhibited. Similarly, the acetylcholine-induced tachycardia was markedly inhibited following oxybutynin. The epinephrine pressor response was enhanced, as was the heart rate response. The systolic/diastolic pressures and heart rate response to norepinephrine were also significantly altered. The respiratory rate response to norepinephrine was slightly, but significantly, increased. Only the diastolic pressure response to isoproterenol was significantly increased following oxybutynin.

Divergent responses due to the "state-of-the-animal" were reported previously (1–3). The influence of differential state on response must be considered when interpreting the experimental data, particularly from the anesthetized preparation.

The differences in systolic/diastolic arterial pressures, heart rates, and respiration rates are summarized in Table V. Basal heart rate was considerably lower in the conscious state, while basal respiratory rate was significantly greater. The amplitude of the changes indicated that the conscious animal exhibits greater oxybutynin sensitivity and response intensity. Greater oxybutynin tachycardic response in conscious animals can be explained on the basis of the Law of Initial Values (8).

In the conscious dog, the oxybutynin response was pressor; following pentobarbital anesthesia, the same oxybutynin doses evoked a systolic/diastolic arterial depressor response. These findings are inexplicable.

The consistent and significant tachycardia produced by oxybutynin in conscious and anesthetized dogs and the selective oxybutynin inhibition of the acetylcholine-induced cardiovascular responses in both conditions support the earlier pharmacological report. Oxybutynin has mild to moderate cardiovascular effects, probably through an anticholinergic mechanism of action.

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Amino Acid Effect on Aspirin Stability in Propylene Glycol

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Received July 6, 1978, from the *School of Pharmacy, West Virginia University, Morgantown, WV 26506.* Accepted for publication November 6, 1978. *Present address: School of Pharmacy, University of Maryland, Baltimore, MD 21201.

Abstract □ Temperature stability studies were conducted on 0.36 *M* (6.5% w/v) aspirin solutions including either 0.02 *M* L-methionine or 0.02 *M* histidine in propylene glycol. Aspirin was determined spectrophotofluorometrically as salicylic acid content at 412 nm. A 0.36 *M* aspirin in polyethylene glycol 400 solution was studied concurrently. Aspirin degradation rate constants, *k*, obtained from semilogarithmic plots of percent drug remaining *versus* time at 30–70 ± 0.5° were used for preparing Arrhenius plots. Good correlation was seen between predicted aspirin stability and experimental $k_{25°}$ values. L-Methionine and histidine markedly reduced aspirin stability.

Keyphrases □ Aspirin—stability in propylene glycol, effect of L-methionine and histidine, temperature □ Stability—aspirin in propylene glycol, effect of L-methionine and histidine, temperature □ Propylene glycol—stability of aspirin solutions, effect of L-methionine and histidine, temperature

Aspirin remains the most sought-after, nonprescription analgesic. It possesses a combination of anti-inflammatory, antipyretic, and analgesic properties unparalleled by other "aspirin-like" compounds. Aspirin is, however, a notoriously unstable drug and degrades to the less potent salicylic acid in the presence of moisture (1). Liquid aspirin dosage forms present a challenging pharmaceutical problem because of this instability in water and in vehicles containing traces of water.

Numerous attempts to stabilize aspirin solutions, especially in nonaqueous solvents such as polyethylene glycol 400 (2), glycerol and propylene glycol (3, 4), ethanol, (5), and esterified polyethylene glycols (6) have been reported. The objective of the present study was to measure

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