Bupropion (Zyban, Wellbutrin) Inhibits Nicotine-Induced Viral Reactivation in Herpes Simplex Virus Type 1 Latent Rabbits

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ABSTRACT

We reported that nicotine applied via a transdermal patch (21 mg/day) induced viral reactivation and ocular shedding in herpes simplex virus type 1 (HSV-1) latent rabbits. One possible mechanism of action involves the release of catecholamines and other similar agents, triggering HSV reactivation. Bupropion (Zyban, Wellbutrin), a non-nicotine aid to smoking cessation, inhibits neuronal uptake of norepinephrine, serotonin, and dopamine. To determine whether bupropion inhibits HSV reactivation, rabbits latent with HSV-1 were grouped (at least 10 rabbits/group) and treated as follows: nicotine patch (transdermal delivery) and bupropion (Zyban sustained-release tablets (150 mg) twice a day (oral)), nicotine patch only, Zyban tablets only (twice a day (oral)), nicotine patch with oral placebo (twice a day (oral)), or no drug treatment. Eyes were swabbed for 22 consecutive days. The appearance of HSV-1 in the tear film was significantly less frequent in the bupropion-treated rabbits, in terms of positive rabbits/total rabbits, positive eyes/total eyes, and positive swabs/total swabs. Nicotine-treated rabbits had 78/440 (17.7%) positive/total swabs, whereas bupropion-treated rabbits had 47/792 (5.9%) positive/total swabs. Thus, bupropion significantly reduces nicotine-induced HSV reactivation in latent rabbits.

Herpes simplex virus type-1 (HSV-1) is a neurotropic virus that belongs to the Herpesviridae family, which includes seven other human viruses and a multitude of animal viruses (Jones, 2003). Along with its cousins, which produce a variety of clinical conditions among farm and companion animals, this alphaherpesvirus is known to cause one of the most common corneal infections of the human eye. The virus typically affects epithelial or mucosal cells of tissues throughout the body and can lead to acute and/or recurrent cases of keratitis, which is generally preceded by the formation of a pathognomonic dendritic lesion in the cornea. Sporadic recurrent HSV reactivation often affects the sensory and autonomic ganglia from which it can convert (reactivate) to a lytic infection (Wagner and Bloom, 1997; Miller et al., 1998). Virions reach the nuclei of ganglionic neurons from their point of entry by retrograde axonal transport, whereby they travel along ciliary nerve tracts and remain in a dormant state until reactivated. Reactivation typically occurs following any number of exogenous or endogenous stimuli including stress, immunomodulation, or direct tissue manipulation (Kwon et al., 1981; Hill et al., 1987; Colgin et al., 2001). In cases of primary ocular infections, HSV-1 has been shown to establish latency within neurons of sensory and autonomic ganglia from which it can convert (reactivate) to a lytic infection (Wagner and Bloom, 1997; Miller et al., 1998). Virions reach the nuclei of ganglionic neurons from their point of entry by retrograde axonal transport, whereby they travel along ciliary nerve tracts and remain in a dormant state until reactivated. Reactivation typically occurs following any number of exogenous or endogenous stimuli including stress, immunomodulation, or direct tissue manipulation (Kwon et al., 1981; Hill et al., 1987; Colgin et al., 2001). In cases of primary ocular infections, HSV-1 has been shown to establish latency within the trigeminal ganglia, which is generally preceded by the formation of a pathognomonic dendritic lesion in the cornea. Sporadic recurrent ocular reactivation causes significant morbidity, including corneal scarring, reduced visual acuity, and in severe cases, blindness (Whitley, 2001).

As the chief alkaloid within tobacco products, nicotine produces its effect by binding stereoselectively to nicotinic acetylcholine receptors (nAChRs) throughout the central and peripheral nervous systems (Thuerauf et al., 1999; Alimohammadi and Silver, 2000; Balfour and Ridley, 2000). Located in presynaptic terminals of neurons throughout the body and can lead to acute and/or recurrent cases of keratitis, which is generally preceded by the formation of a pathognomonic dendritic lesion in the cornea. Sporadic recurrent HSV reactivation often affects the sensory and autonomic ganglia from which it can convert (reactivate) to a lytic infection (Wagner and Bloom, 1997; Miller et al., 1998). Virions reach the nuclei of ganglionic neurons from their point of entry by retrograde axonal transport, whereby they travel along ciliary nerve tracts and remain in a dormant state until reactivated.
aptic ganglia of both branches of the autonomic nervous system, the adrenal medulla, neuromuscular junctions, and the brain, neuronal nicotinic receptors are prototypic ionotropic receptors that consist of three \( \alpha \)-subunits and two \( \beta \)-subunits or some combination thereof (Anand et al., 1991; Cooper et al., 1991; Liu et al., 1998). When activated by nicotine, these receptors initiate a cascade of events that promote the release of acetylcholine, norepinephrine, dopamine, and adrenocorticotropic hormone (ACTH), resulting in prominent excitatory and inhibitory responses by the nerves that modulate the activity of effector organs (Matta et al., 1990, 1998; Di Chiara, 2000; Dani and De Biasi, 2001; Miller et al., 2002).

We have previously shown that nicotine can induce HSV-1 reactivation in rabbits latently infected with HSV-1 strain McKrae (Myles et al., 2003). Our present study uses systemic bupropion (Zyban-sustained-release tablets given orally) to determine whether this agent can block the nicotine-induced HSV reactivation. Bupropion (Zyban-SR; GlaxoSmithKline, Uxbridge, Middlesex, UK) is a novel antidepressant agent that has been used as a non-nicotine drug to aid in smoking cessation (Ascher et al., 1995; Hurt et al., 1997; Jorenby et al., 1999). Bupropion’s mechanism of action is believed to involve inhibition of neuronal uptake of norepinephrine, dopamine, and serotonin (Cooper et al., 1994; Ascher et al., 1995; Tella et al., 1997; Miller et al., 2002). Ancillary evidence suggests a significant reduction in HSV recurrences (reactivation) in patients taking bupropion (Zyban-SR) sustained-release tablets.

**Materials and Methods**

**Animals, Viruses, and Cells.** New Zealand white rabbits weighing between 2 and 3 kg and of both sexes were selected for inoculation. All animals were handled and maintained in accordance with the tenets established by the National Institutes of Health on the care and use of animals in research. The virus used in this experiment was HSV-1 strain McKrae. Viral stocks were propagated in flasks containing a confluent monolayer of primary rabbit kidney cells in Earle’s minimal essential medium with 10% fetal calf serum at a multiplicity of infection of 0.01. Viral titers were determined with the use of African green monkey (CV-1) cells.

**Rabbit Inoculation and Randomization.** Rabbits were bilaterally inoculated with 2.5 \( \times 10^6 \) pfu of HSV-1 strain McKrae in a 25-\( \mu \)l suspension. Prior to inoculation, corneas were scarified in a 4 \( \times \) 4 crosshatch pattern. Selected virus was then placed into the lower conjunctival cul-de-sac of each eye and the lower eyelid gently massaged over the eye for 30 s. Acute infection was confirmed by slit lamp biomicroscopy on postinoculation days 4 to 6. Rabbits were equally divided into groups based on slit lamp scores taken on postinoculation day 29.

**Nicotine Administration.** Systemic delivery of nicotine was administered to selected rabbits by transdermal patches purchased from Schein Pharmaceutical (Florham Park, NJ). The patches used in this experiment provided an in vivo delivery rate of 21 mg/day. Beginning on postinoculation day 30 and continuing daily for 20 days, rabbits chosen to receive nicotine had a single patch placed on alternating ears. Patches were held in place by zinc oxide tape. Zinc oxide-coated medical tape was applied to the ears of controls.

**Bupropion Administration.** Bupropion (Zyban-SR) was given once daily for 3 days then twice daily for 20 consecutive days. The Zyban-SR tablets contained 150 mg of bupropion hydrochloride in a sustained release form. There was no observable toxicity for rabbits that received either the nicotine transdermally or the bupropion orally for the 20 days of experiments. The placebo control for Zyban-SR was oral delivery of inert tablets.

**Ocular Swabbing.** Each rabbit eye was swabbed daily for 22 days beginning on the 1st day of nicotine treatment. Tear film was collected by placing a sterile Dacron-tipped swab into the lower conjunctival cul-de-sac of the eye, with care taken to avoid touching the cornea. Swabs were placed directly into a primary rabbit kidney cell culture containing Earle’s minimal essential medium and 2% fetal calf serum and removed after an incubation period of 48 h at 37°C in a 5% CO\(_2\) atmosphere. Cells were monitored every other day for 10 days for cytopathic effect. Blind passage was performed on all cultures that did not show cytopathic effect.

**Serum Collection.** On the final day of nicotine administration, blood was collected from all rabbits for subsequent analysis by mass spectrometry to determine serum nicotine concentrations. Rabbits receiving nicotine had samples collected just prior to patch replacement (0 h) and at postreplacement h 6. Rabbits not receiving nicotine had one sample each drawn to serve as baselines. An average of 2 to 3 ml of whole blood was retrieved each time by placing a 27-gauge needle into the marginal ear vein of the ear without the transdermal patch. Collected blood was placed into sterile Vacutainer tubes and centrifuged at 2000 rpm for 5 min. Serum was withdrawn and transferred to another set of sterile Vacutainer tubes and stored at \(-70^\circ\)C prior to analysis.

**Nicotine, Cotinine, Bupropion, and Hydroxy-Bupropion Measurement.** To each serum sample (0.5 ml) was added 50 \( \mu \)l of a 1.0 \( \mu \)g \( d_3 \)-cotinine (MeOH; Cerilliant, Round Rock, TX) solution as internal standard. The pH of the samples was adjusted to 10 by the addition of 0.5 ml of sodium hydroxide (0.1 N), after which 3.0 ml of n-butyl chloride was added. The samples were mixed (rotorack) for 10 min and centrifuged for 10 min to separate the layers. The upper organic phase was transferred to a 5-ml conical tube and 3.0 ml of 1.0 N sulfuric acid was added. Mixing and centrifuging steps were repeated as before. The upper layer was aspirated to waste, and the lower aqueous layer was transferred to a 5-ml conical tube. Concentrated ammonium hydroxide (0.5 ml) was added along with 5.0 ml of dichloromethane. Mixing and centrifuging were repeated. The upper aqueous layer was aspirated to waste, and the lower dichloromethane layer was transferred to a 5-ml conical tube. The solvent was removed in a hood using a stream of dry nitrogen gas. Standard curves were prepared by spiking reference materials for cotinine (Cerilliant), nicotine, bupropion, and hydroxybupro- pion (Alltech-Applied Science Labs, State College, PA), as well as \( d_3 \)-cotinine as the internal standard, into liquid chromatography (LC) grade water followed by extraction, as described above. Method blanks and serum blanks, with no internal standard, were similarly processed. All solvents were obtained from commercial sources and were LC grade or better. The samples were reconstituted with 150 \( \mu \)l of 50:50 0.1% formic acid (pH 6.0):acetonitrile, filtered with a 0.2-\( \mu \)m syringe filter (Nalgene/nylon) and analyzed by absorption photometry chemical ionization liquid chromatography/mass spectrometry/mass spectrometry (MicroMass Quattro II LC/MS/MS, Beverly, MA) using the heated nebulizer in the positive ion mode. Multiple (three) daughter ion transitions (collision-induced dissociation using argon gas) for each compound were monitored in the multiple reaction monitoring mode.

Chromatography was performed using an Alltech Alltima C18, 5 \( \mu \)m, 3.2- \( \times \) 150-mm column with a gradient mobile phase consisting of 0.1% formic acid, pH 6.0 (A) and acetonitrile (B): 0 to 1 min, 95% A and 5% B; 7 min, 100% B; 10 min, 100% B; and 18 min, 95% A and 5% B. The standard curves for bupropion and hydroxybupropion were in the range of 1.0 to 100 ng/ml and for nicotine and cotinine 1.0 to 2,000 ng/ml, based on 0.5 ml of sample. Standard curves were plotted as a function of spiked concentration versus the area ratio of the corresponding compound’s ion mass peak to that of the internal standard. The linearity of the curve \( r^2 \) and the equation of the line were derived for determination of sample concentrations. The drug levels were analyzed using a two-way analysis of variance with time (0 and 6 h) and treatments (five treatment combinations) as main effects and drug level as the outcome. Means of time and treatment combinations were compared with protected \( t \) tests, with the exper-
iment-wise alpha level maintained at 0.05 using a simulation method (Edwards and Berry, 1987).

Results

Effect of Bupropion on Ocular Shedding of HSV-1. Table 1 shows the swab results obtained from rabbits latently infected with HSV-1 strain McKrae. The results show that nicotine administration significantly increases reactivation, compared with controls (swabs positive/total swabs; 17.7% versus 11.1%). The difference was significant ($p = 0.0019$, Fisher’s exact test). Rabbits latent with HSV-1 McKrae and treated with nicotine had a significantly higher number of episodes, compared with the controls (data not shown). Interestingly, bupropion (Zyban-SR) reduced the level of spontaneous shedding to ~50% of the level seen in control rabbits. Table 2 shows the swab data obtained following concurrent administration of bupropion and nicotine. Bupropion inhibited nicotine-stimulated ocular shedding of HSV-1 from latent rabbits. There is no significant difference between the level of stimulation obtained when bupropion was administered alone (5.2%, Table 1) or in combination with nicotine (5.9%) ($p > 0.05$). Compilation of data from separate experiments demonstrated that 17.4% (485/2782) of the swabs taken from rabbits treated with nicotine (five experiments) were positive for virus, compared with only 9.5% (102/1079) of swabs taken from controls (four experiments). Rabbits receiving nicotine exhibited a significantly higher rate of HSV-1 ocular shedding than controls (Table 1; $p = 0.0071$ by Fisher’s exact test). However, rabbits receiving bupropion (either alone or with nicotine) had a significantly lower rate of ocular shedding (5.7%; 70/1232; Table 1 and Table 2), compared with controls ($p = 0.0001$ by Fisher’s exact test).

Metabolism of Nicotine and Bupropion. Serum levels of nicotine, bupropion, and their major metabolites were determined in rabbits that received both drugs. Serum levels of both drugs were increased in all rabbits 6 h following patch replacement or oral administration of bupropion (Table 3). Standard curves for nicotine, cotinine, bupropion, and hydroxybupropion were linear over the range examined and determined for the samples ($r^2$ for nicotine = 0.997, cotinine = 0.998, bupropion = 0.992, and hydroxybupropion = 0.999). Although there is variability in the total serum levels of drug, we have not detected a difference in percentage stimulation of ocular shedding by nicotine or the ability of bupropion (Zyban-SR) to inhibit the nicotine stimulated reactivation of latent HSV-1 (unpublished observations). The levels of cotinine (major metabolite of nicotine) and of hydroxybupropion (major metabolite of bupropion) were also increased 6 h following administration of the parent compounds. Analysis (protected $t$ test) of the mean values for the treatment time combinations showed that: the level of drug (nicotine or cotinine) did not differ significantly between its initial baseline value and that obtained at 6 h postpatch replacement, and the drug levels in individual rabbits differed significantly from one another at the 6-h time point.

Discussion

The present study demonstrates that bupropion inhibits nicotine-stimulated ocular shedding of HSV-1 in latent rabbits. The results also indicate that bupropion alone reduces the frequency of spontaneous ocular shedding by ~50%, compared with placebo controls. This inhibitory effect of bupropion on spontaneous HSV-1 reactivation is observed in the presence or absence of nicotine. The effect of bupropion on spontaneous, recurrent HSV-1 ocular shedding tends to support the anecdotal evidence of a reduction in the frequency and/or severity of recurrent herpetic disease in individuals taking Zyban sustained-release tablets. In these studies, bupropion treatment lasted for 3 weeks. Since steady-state levels of bupropion and/or its metabolites would be expected to be achieved in <2 days (Hsyu et al., 1997), extension of treatment (>3 weeks) would not necessarily translate into a continued decline in number/frequency of spontaneous, recurrent HSV-1 lesions or episodes.

There are two possible mechanisms to explain the effect of bupropion on spontaneous HSV-1 ocular shedding. First, bupropion, an atypical antidepressant, inhibits neuronal uptake of norepinephrine, serotonin, and dopamine (Ascher et al., 1995) but does not inhibit the monoamine oxidases involved in their breakdown (Fowler et al., 1996). Thus, an overall decrease in levels and/or uptake of these neurotransmitters, especially norepinephrine, could account for the reduction in spontaneous, recurrent HSV-1 lesions in bupropion (Zyban)-treated individuals. Second, chronic administration of bupropion (and other antidepressant drugs) has been reported to decrease the expression of tyrosine hydroxylase, the rate-limiting enzyme in synthesis of norepinephrine (Nestler et al., 1990). A reduction (~40%) in levels of tyrosine hydroxylase mRNA and protein would indicate that the synthesis of norepinephrine may also be decreased during chronic bupropion administration. Catecholamines (norepinephrine and epinephrine) have been shown to stimulate HSV-1 reactivation (Kwon et al., 1981; Hill et al., 1987). Therefore, the observed effects of bupropion on spontaneous HSV-1 ocular shedding may be attributed to reduced neuronal uptake of norepinephrine and a concurrent reduction in synthesis of norepinephrine due to decreased expression of the hydroxylase enzyme.

Systemically administered nicotine is thought to act within the brain to stimulate the release of ACTH (Matta et al., 1990, 1998), norepinephrine, dopamine, and acetylcholine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rabbits Positive/Total Rabbits</th>
<th>Eyes Positive/Total Eyes</th>
<th>Swabs Positive/Total Swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (orally)</td>
<td>7/10 (70%)</td>
<td>13/20 (65%)</td>
<td>49/440 (11.1%)</td>
</tr>
<tr>
<td>Bupropion</td>
<td>6/10 (60%)</td>
<td>9/20 (45%)</td>
<td>23/440 (5.2%)**</td>
</tr>
<tr>
<td>Nicotine (patch)</td>
<td>10/10 (100%)</td>
<td>20/20 (100%)</td>
<td>78/440 (17.7%)**</td>
</tr>
</tbody>
</table>

* $p = 0.0019$ compared with placebo controls (Fisher’s exact test).

** $p = 0.0071$ compared with placebo controls (Fisher’s exact test).
TABLE 2
Ocular shedding of HSV-1 strain McKrae by nicotine- and nicotine plus bupropion-treated rabbits

New Zealand white rabbits were inoculated bilaterally with 2.5 × 10^5 pfu of HSV-McKrae into scarified (4 × 4 crosshatched) eyes. Following establishment of latency (postinoculation day ≥28), nicotine was administered systemically to selected rabbits by daily application of transdermal patches (21 ng/day in vivo delivery rate) to alternating ears for 20 days. Starting on day 1 of nicotine patch administration, bupropion was administered twice daily for the duration of the experiment. All eyes were swabbed daily for 22 days after the beginning of treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rabbits Positive/Total Rabbits</th>
<th>Eyes Positive/Total Eyes</th>
<th>Swabs Positive/Total Swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine patch + placebo (orally)</td>
<td>18/18 (100%)</td>
<td>35/36 (97.2%)</td>
<td>149/792 (18.8%)</td>
</tr>
<tr>
<td>Nicotine patch + bupropion</td>
<td>7/18 (39%)</td>
<td>13/36 (36.1%)</td>
<td>47/792 (5.9%)</td>
</tr>
</tbody>
</table>

*p = 0.0001 compared with nicotine/placebo (Fisher’s exact test).

### TABLE 3
Serum levels of nicotine, cotinine, bupropion, and hydroxybupropion following patch replacement

On the final day of treatment (bupropion with or without nicotine), blood was collected from all rabbits to determine serum nicotine, cotinine, bupropion, and hydroxybupropion concentrations. Blood was drawn just prior to nicotine patch replacement or oral administration of Zyban sustained-release tablets (0 h) and 6 h after patch replacement or administration of bupropion. Since the nicotine transdermal patches were replaced every 24 h (at 8:30 AM), time 0 was equivalent to a baseline value (24 h postpatch replacement). Serum levels of nicotine, bupropion, and their major metabolites were measured as described under Materials and Methods using gas and LC/mass spectrometry techniques. G-13, -15, -16, -19, and -20 denote individual rabbits.

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Nicotine 0 h</th>
<th>Nicotine 6 h</th>
<th>Cotinine 0 h</th>
<th>Cotinine 6 h</th>
<th>Bupropion 0 h</th>
<th>Bupropion 6 h</th>
<th>Hydroxybupropion 0 h</th>
<th>Hydroxybupropion 6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-13</td>
<td>427</td>
<td>644</td>
<td>49</td>
<td>89</td>
<td>0</td>
<td>33</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>G-15</td>
<td>822</td>
<td>1020</td>
<td>176</td>
<td>524</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>1.1</td>
</tr>
<tr>
<td>G-16</td>
<td>322</td>
<td>335</td>
<td>345</td>
<td>582</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>G-19</td>
<td>180</td>
<td>523</td>
<td>231</td>
<td>359</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>G-20</td>
<td>522</td>
<td>817</td>
<td>452</td>
<td>377</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>1.8</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>454.6 ± 98.5</td>
<td>667.8 ± 107.7</td>
<td>250.6 ± 63.3</td>
<td>386.2 ± 78.1</td>
<td>0.23 ± 0.23</td>
<td>0.92 ± 0.23</td>
<td>0.92 ± 0.23</td>
<td>0.23 ± 0.23</td>
</tr>
</tbody>
</table>

(Summers and Giacobini, 1995; Wonnacott, 1997). Evidence indicates ACTH and catecholamines (norepinephrine and epinephrine) have a role in the stress response (Matta et al., 1998), and dopamine is thought to be a prominent player in the addictive properties of nicotine and other drugs of abuse (Balfour and Ridley, 2000; Di Chiara, 2000; Schoffelmeer et al., 2002). Nicotine initiates its action by binding to nAChRs that are widely distributed throughout the mammalian central nervous system (Liu et al., 1998; Thuerauf et al., 1999; Dani and De Biasi, 2001). Thus, we (Myles et al., 2003) hypothesize that the effect of nicotine on HSV-1 ocular shedding is most likely due to a summation of diverse responses initiated by nicotine binding to the nAChR.

Bupropion’s inhibition of nicotine-stimulated HSV-1 ocular shedding is in accord with its usage as a non-nicotine aid to smoking cessation (Fryer and Lukas, 1999; Slemmer et al., 2000). Miller et al. (2002), using functional neurotransmitter release assays, demonstrated that bupropion acts as an antagonist at α3β2 and α3β4 nAChRs in rat striatum and hippocampus, respectively. Interestingly, bupropion concentrations required for inhibition of neurotransmitter (norepinephrine and dopamine) release were in the same concentration range that has been reported to inhibit norepinephrine and dopamine transporters (Ascher et al., 1995).

The metabolism of nicotine has been studied extensively. Benowitz et al. (1994) reported that in humans: on average, 88% of a systemic dose of nicotine can be accounted for by measurement of nicotine and its metabolites; on average, 70% of nicotine is converted to cotinine, a major metabolite; the pattern of metabolism is similar whether nicotine is inhaled or absorbed transdermally; and although there is considerable variation among individuals, the pattern of metabolism is consistent for an individual. In our previous report (Myles et al., 2003), we observed a similar degree of variation when the total serum levels of nicotine were determined following transdermal delivery to rabbits. In that study, however, we did not determine serum cotinine concentration. As can be seen from Table 3, serum levels of cotinine are variable, thus indicating a wide variation in nicotine metabolism in rabbits. It has been shown that cotinine does not have a significant effect on the kinetics or disposition of nicotine (Benowitz and Jacob, 1993; Benowitz et al., 1994; Zevin et al., 1997). Thus, even with high steady-state levels of cotinine observed in smokers (Hsyu et al., 1997; Zevin et al., 1997) or in rabbits (Table 3), the kinetics of nicotine metabolism would not be expected to be significantly altered. To our knowledge, these are the first reports that examine the metabolism of nicotine in the rabbit.

The metabolic disposition of bupropion has also been examined (Hsyu et al., 1997, and refs. therein). A single 150-mg
bupropion sustained-release tablet was administered to smokers and nonsmokers. The pharmacokinetic parameters were then calculated for bupropion and three major metabolites (hydroxybupropion, threohydrobupropion, and erthro-hydrobupropion, with the latter two expressed as a composite total). The mean peak concentration ($C_{\text{max}}$) values for bupropion were 144 ± 28 and 143 ± 39 ng/ml for smokers and nonsmokers, respectively. The half-life ($t_{1/2}$) of bupropion was 19 ± 5 and 18 ± 3 h for smokers and nonsmokers, respectively. In summary, no clinically significant differences between smokers and nonsmokers were observed for the metabolic fate(s) of bupropion. Extrapolation of the results of our study is not helpful due to the paucity of information on the metabolism of bupropion and nicotine in the rabbit. However, our data suggest that both bupropion and nicotine are metabolized with similar kinetics.

The ability of nicotine to stimulate ocular shedding of HSV-1 in the latent rabbit is supported by reports of functional nAChRs in the trigeminal ganglion (site of HSV-1 latency) and/or nerve endings. Liu et al. (1998) used reverse transcriptase polymerase chain reactions and immunocytochemical techniques to identify neuronal nAChRs in the rat trigeminal ganglia. The results demonstrated that rat trigeminal ganglion neurons contain the entire spectrum (α2–α7, α9, and β2–β4) of mammalian neuronal nAChR subunits. Neuronal nAChRs are mainly composed of five subunits in some combination of α (α2–α6) and β (β2–β4) subunits (Anand et al., 1991; Cooper et al., 1991). Evidence for the presence of neuronal nAChRs in the periphery has also been reported (Alimohammadi and Silver, 2000). Administration of classic nAChR blockers (mecamylamine or dihydro-β-erythroidine) blocked the ability of the ethmoid nerve to respond to vapor-phase nicotine. These blockers had no effects on ethmoid nerve responses to cyclohexanone. Based on these results and the known specificity of dihydro-β-erythroidine and mecamylamine for nAChRs, it is concluded that nicotinic receptors, specifically α3β4 and α4β2 subtypes, are expressed in the nasal cavity, presumably on trigeminal free-nerve endings. These nAChRs subunits, identified in the rat trigeminal ganglion (Liu et al., 1998) and demonstrated in rat peripheral trigeminal free-nerve endings (Alimohammadi and Silver, 2000), may equate to the receptors involved in nicotine perception by the trigeminal system in humans (Thuerauf et al., 1999).

Future studies will examine the effect of other nAChR antagonists such as mecamylamine and dihydro-β-erythroidine or selected agonists such as epibatidine and cytisine on nicotine-stimulated ocular shedding of HSV-1 in latent mice and rabbits.

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References


