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Differences in the Receptor Binding Profile of Lofexidine Compared to Clonidine

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Abstract

Lucemyra® (lofexidine hydrochloride) has recently been approved by the US FDA for the mitigation of withdrawal symptoms to facilitate abrupt discontinuation of opioids in adults. Lofexidine is an alpha-2 adrenoceptor agonist. However, the clinical attributes of lofexidine differ in advantageous ways from the classical alpha-2 adrenoceptor agonist clonidine. In the present study, we measured the receptor binding profile of lofexidine and clonidine in an effort to gain an insight into the clinical difference(s).

Keywords

Lofexidine, Clonidine, Opioid Withdrawal, Receptor Affinity, Alpha-2 Agonist

1. Introduction

The current "opioid crisis" has generated a renewed interest in evaluating the benefit-risk balance in patients' administered opioids appropriately, or persons' taking opioids as part of an opioid abuse disorder (OUD) [1]. This type of evaluation has increasingly resulted in the recommendation that the patients reduce their opioid regimen, or even eliminate it. In some cases, a patient may choose to voluntarily taper their opioid medication [2]. In other cases, a person who has an OUD might have to face the reality of an abstinence imposed upon them by the legal system [3]. In both cases, despite agreement about reducing opioid intake, fear of the unpleasant and distressing physiological sequelae that occur during withdrawal inhibits compliance [4]. Fear of the stigma, even of good-intentioned efforts, is also a barrier [5]. Thus, fear of withdrawal is a strong motivating factor

for resisting, or even avoiding, opioid taper and/or discontinuation [6] [7].

For this reason, pharmacotherapy that might help ameliorate withdrawal symptoms during either voluntary or an imposed withdrawal can serve not only a beneficial physiological function, but might also be helpful in a psychological sense, offering the individual a more optimistic view of the decision to taper their legitimate or illegitimate opioid use. In the former case, this could assist in transition to non-opioid analgesics or non-pharmacologic treatment, and in the latter case could make the decision to seek treatment a little easier.

Traditional non-opioid pharmacologic treatment to ameliorate symptoms of opioid withdrawal has mainly involved the off-label use of clonidine. This is because there is a large release of norepinephrine during withdrawal from opioids (**Figure 1**) [8] [9] [10]. Alpha-2 adrenoceptor agonists bind to presynaptic alpha-2 adrenoceptors and inhibit the release of norepinephrine. Clonidine is an alpha-2 adrenoceptor agonist [11]. Lofexidine is also an alpha-2 adrenoceptor agonist, but lofexidine's clinical attributes differ in several advantageous ways from clonidine [12]. The reason might be explained by differences in receptor binding.

In the present study, we report the binding affinity of lofexidine and clonidine in a variety of *in vitro* assays. In the assays in which the drugs were active, they displayed agonist activity (as determined by *in vitro* functional assays).

2. Methods

Lofexidine hydrochloride and clonidine hydrochloride were studied in a broad screen of receptor binding, uptake, and functional assays by Eurofins Scientific. Vectors containing the receptors were transfected into HEK293 (human embryonic kidney 293) cells, and the assays were conducted according to methods previously published [13]-[19].

The receptor binding affinity of compounds was calculated as the percent

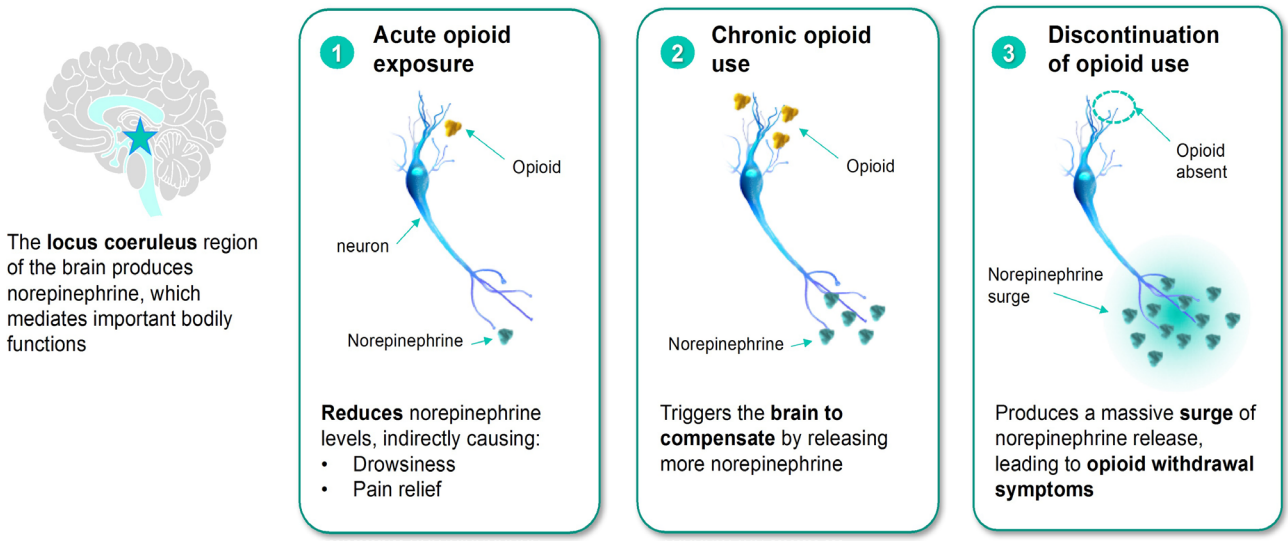

Figure 1. Pictorial representation of the effect of acute and prolonged opioid exposure on norepinephrine in the locus cœruleus.

inhibition of the binding of a radioactively labeled ligand specific for each of the targets. Functional cellular agonist effect (efficacy) was calculated as percent of control response to a known reference agonist. A difference greater than 50% is considered significant in these assays.

EC50 values (the concentrations of test compounds estimated to produce a half-maximal response in an assay) were determined by non-linear regression analysis of the concentration-response curves that were generated, with mean replicate values, using Hill equation curve fitting [20].

3. Results

Clonidine and lofexidine were inactive in kappa opioid receptor assay (rat recombinant CHO cells, 0.3 μM U-50488, 10 min, 37˚C), 5-HT_{2A} (human recombinant HEK-293 cells, 10 μM serotonin, 30 min, 37˚C), 5-HT_{2B} (human recombinant CHO cells, 1 μM serotonin, 30 min, 37˚C), 5-HT transporter (human recombinant CHO cells, 2 nM imipramine, 60 min, room temperature), and MAO-A (rat cerebral cortex, 10 nM Ro-41-1049, 60 min, 37˚C). Clonidine displayed significant binding affinity and agonist functional activity (pEC50 ≥ 5 M) at the following receptors: alpha-adrenoceptor sites: alpha-1A, alpha-2A, and alpha-2C (**Figure 2**). It was devoid of significant activity (agonist or antagonist) at the other sites examined.

Lofexidine displayed significant binding affinity and agonist functional activity (*viz.*, pEC50 ≥ 5 M) at alpha-adrenoceptor receptors alpha-1A, alpha-2A, alpha-2B, and alpha-2C. In addition to the activity at alpha-adrenoceptors, lofexidine also displayed significant agonist activity at dopamine D_{2S}, serotonin 5-HT_{1A}, and 5-HT_{1B} (**Figure 3**).

The receptor binding affinity of lofexidine at these sites is shown in **Figure 4**. Similar to the profile of clonidine, lofexidine has affinity for several alpha-adrenoceptor subtypes. But unlike clonidine, lofexidine has affinity for other receptors, including 5-HT_{1A}, 5-HT_{1B}, and dopamine D_{2S}.

The chemical structures of lofexidine (2-[1-(2,6-Dichlorophenoxy)ethyl]-4,5-dihydro-1*H*-imidazole) and of clonidine (*N*-(2,6-Dichlorophenyl)-4,5-dihydro-1*H*-imidazol-2-amine) are shown in **Figure 5**.

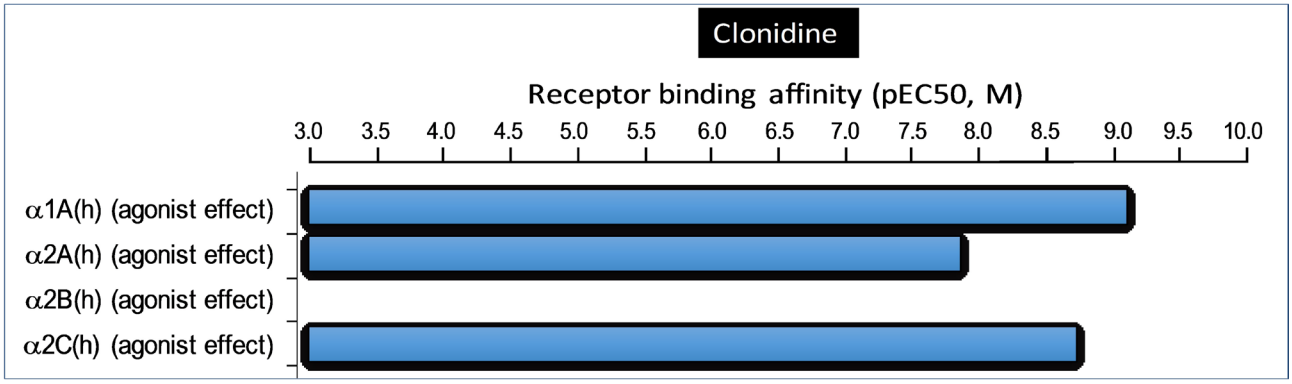

Figure 2. Summary of receptor binding profile of clonidine.

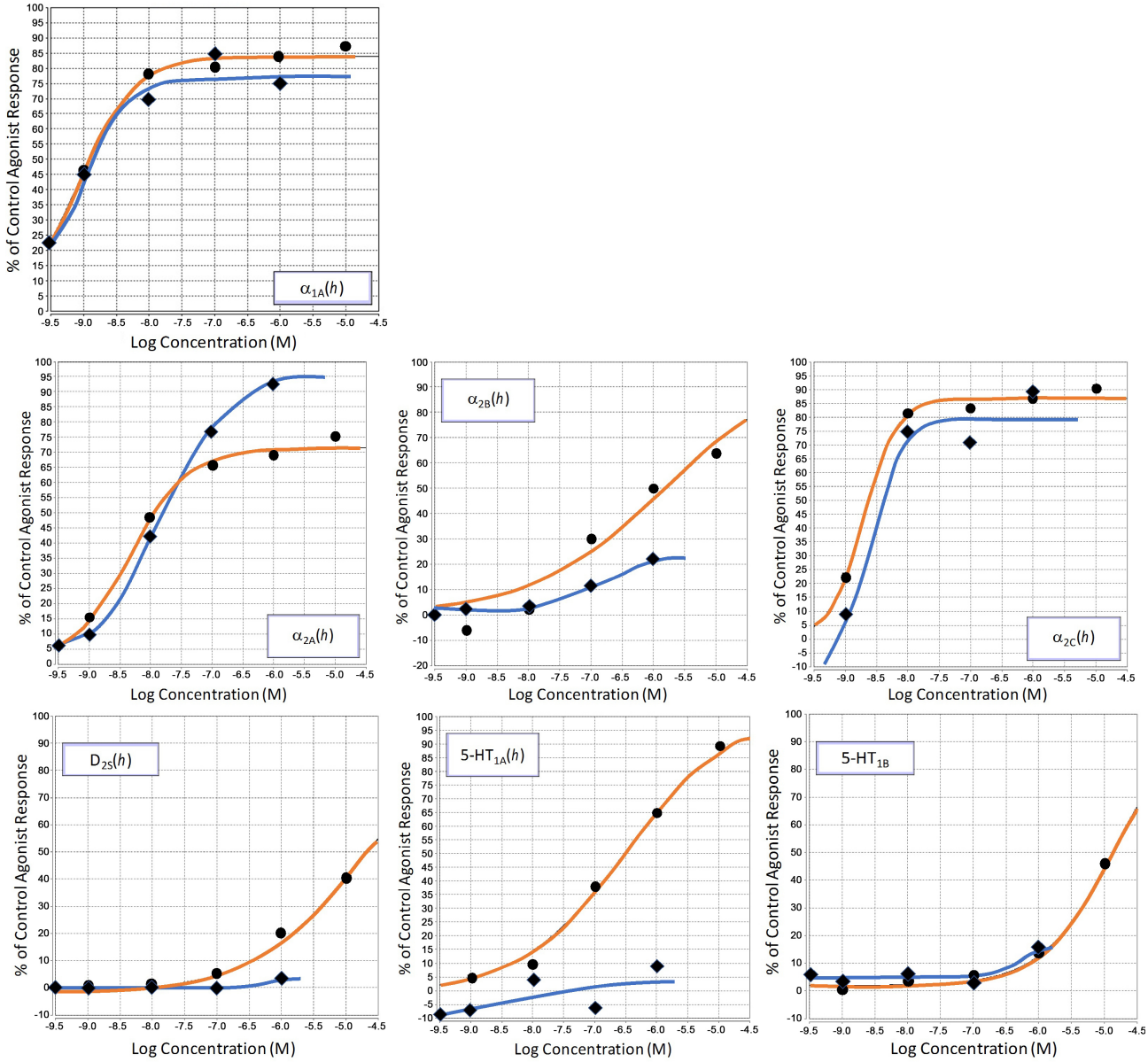

Figure 3. Dose-response curves of lofexidine (dots) and clonidine (triangles) in *in vitro* assays.

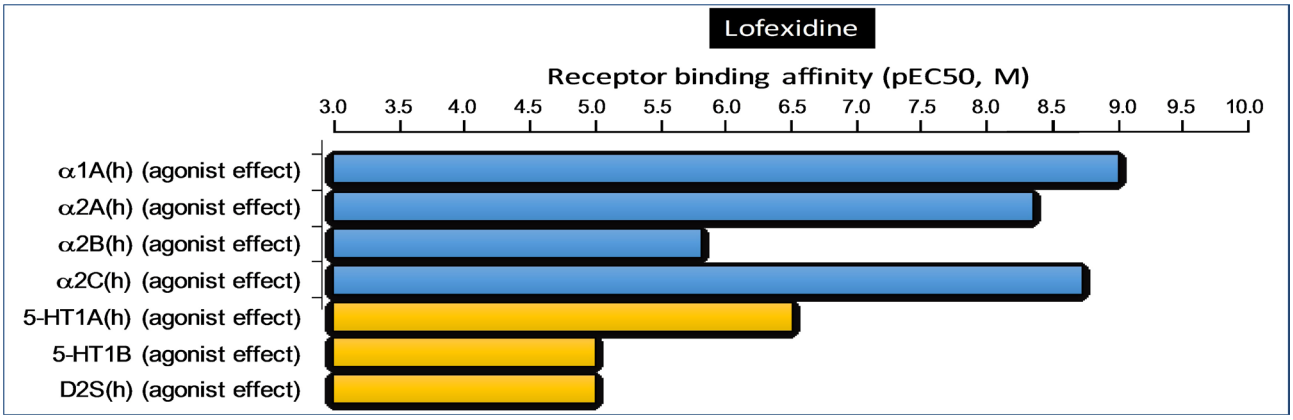

Figure 4. Summary of receptor binding profile of lofexidine.

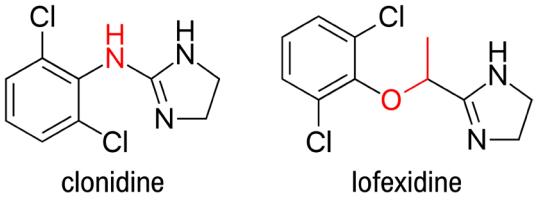

Figure 5. Chemical structure comparison of lofexidine and clonidine.

4. Discussion

For a significant number of people the fear of experiencing negative symptoms during voluntary or forced withdrawal from opioid therapy or abuse is a strong deterrent to seeking help or to compliance to effective tapering regimens, and the combination of uncomfortable symptoms and craving makes completion of withdrawal difficult [21] [22] [23]. Thus, a treatment that can lessen opioid withdrawal symptoms used in conjunction with other treatment modalities results in better outcomes [24].

Based on several studies in rodents and monkeys suggesting that norepinephrine is involved in opioid withdrawal [25] [26] [27] [28] [29], Gold *et al.* (1978) [30] reported early successful experience with the use of the alpha-2 adrenoceptor agonist clonidine to treat symptoms of opioid withdrawal in five patients, followed by a double-blind, placebo-controlled, cross-over trial involving 11 participants [31]. Washton *et al.* (1980) [32] [33] also reported positive results with clonidine. Other treatments that also decrease noradrenergic tone, such as beta-adrenoceptor antagonism [34] [35] and inhibition of norepinephrine synthesis [36] [37] have been reported to have positive effects on opioid withdrawal symptoms, but they are not as potent on locus cœruleus inhibitory effects or in blocking the physiological effects of heightened noradrenergic activity in clinical doses. Hence, the special value of an alpha-2 adrenoceptor agonist action stands out. However, although clonidine has been effective, it has consistently been reported to be associated with adverse events, in particular, significant changes (drops) in blood pressure [38] [39] [40].

Lofexidine (racemate of *R*-(+)- and *S*-(–) enantiomers, dexlofexidine and levlofexidine, respectively) is a full agonist at alpha-2 adrenoceptors [41] [42] [43] [44] [45]. It was originally reported to be effective in easing the symptoms in opioid-dependent patients by Washton *et al.* in 1981 [46]. It was approved for use in opioid detoxification in the United Kingdom, most of the European Union, and other countries in the 1980s and 1990s. Its efficacy in amelioration of opioid withdrawal symptoms has subsequently been demonstrated in several studies, and according to a recent Cochrane review [24], with fewer adverse effects and better safety profile than clonidine. The same conclusion was reached in an earlier review by Gowing *et al.* (2002) [47]. Notably, the lower incidence of hypotension has been highlighted as the basis for distinguishing lofexidine from clonidine for use in treating the symptoms of opioid withdrawal, and for making it more suitable for this use in outpatient settings [47].

The present study reveals another interesting difference, at the receptor level, between lofexidine and clonidine. Whereas both of the drugs bind to alpha-adrenoceptors, lofexidine displays additional binding that clonidine does not: most notably to 5-HT_{1A} receptors. 5-HT_{1A} receptors have independently been linked to opioid withdrawal. 5-HT levels decrease during withdrawal from opioids [48]. And both opioid withdrawal and post-withdrawal stress induce differential alterations in mRNA expression for genes that regulate 5-HT located in DRN (serotonergic dorsal raphe nucleus) of rats [49]. 5-HT_{1A} receptors are located on cell bodies and dendrites of DRN neurons and they function to maintain 5-HT homeostasis by regulating 5-HT synthesis and release [50] [51]. A compound with 5-HT_{1A} agonist properties has been recently reported to decrease the expression of morphine withdrawal symptoms in opioid-dependent mice [52] [53].

5. Perspective and Conclusions

For a significant number of people, the fear of experiencing negative symptoms during withdrawal from opioids deters compliance with taper regimens and seeking treatment opportunities. Amelioration of withdrawal symptoms results in improved outcomes. The alpha-2 agonist lofexidine has been used for this purpose in several countries for many years, and has recently been approved in the US It is as effective as the standard alpha-2 agonist clonidine, but has a better safety profile. The present study potentially opens a window into a mechanistic understanding of the differentiating attributes of lofexidine and clonidine. Interestingly, the results of this study reveal that lofexidine, but not clonidine, has affinity for 5-HT_{1A} receptors. This is particularly interesting in light of studies demonstrating: 1) changes in 5-HT_{1A} mRNA during withdrawal, and 2) the decreased expression of morphine withdrawal symptoms in mice administered a compound having 5-HT_{1A} agonist activity.

Based on the above, it is intriguing to speculate that a drug that has dual agonist action at alpha-2 adrenoceptors and anxiolytic 5-HT_{1A} receptors will be more effective in treating opioid withdrawal symptoms at doses that produce less adverse effects than single-mechanism alpha-2 agonists.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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