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On deriving the dose–effect relation of an unknown second component: An example using buprenorphine preclinical data

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ABSTRACT

Buprenorphine, like many other drugs, displays a biphasic dose–response relation ('hormesis'), *viz.*, its antinociceptive effect in some preclinical models increases up to some dose level (often achieving 100% effect) and decreases at high-doses. A decreasing component was evident in the tail-flick tests described here, occurring in both the mouse and the rat. While the mechanism of dose-related decline in antinociceptive effect, when observed, might be related to nociceptin/orphanin-FQ, the precise mechanism remains unknown. Regardless of the mechanism, the values of this dose-related decline yield data that can be used to calculate the dose–effect relation of the decreasing (unknown second) component. The calculation, which uses the same concept of dose equivalence that underlies additivity in isobolographic analysis, was employed here from tail-flick data obtained in mouse and rat. The derived dose–effect curves of the second component, though differing in efficacy between mouse and rat, displayed a very notable similarity. This novel technique offers possible insight into the dual low-dose (analgesic), high-dose (addiction medication) uses of buprenorphine.

1. Introduction

Buprenorphine, first synthesized in the late 1960s, is a centrally-acting analgesic that is gaining prominence due to its approved use in opioid addiction treatment. It is an analog of the poppy-derived alkaloid thebaine and possesses high binding affinity for opioid receptors (Villiger and Taylor, 1981; Rothman et al., 1995; Huang et al., 2001; Lutfy and Cowan, 2004). Buprenorphine shares certain preclinical and clinical attributes of standard opioid analgesics such as morphine and fentanyl, but differs by having slow receptor dissociation kinetics, less respiratory depression and immune suppression, and a biphasic ('bell'- or 'inverted U'-shaped) dose–response relation in certain animal models such as the hot water-immersion tail-flick test (reviewed in Cowan and Lewis, 1995 and Budd and Raffa, 2005). Such a biphasic dose–response curve ('hormesis') is actually more common than generally recognized (Calabrese, 2008). This was strikingly evident in a recent study in mouse (Raffa and Ding, 2007), which showed that the dose–effect curve reached 100% maximum possible effect, but was biphasic, increasing at doses below 10 mg/kg and decreasing at doses above 10 mg/kg. While the precise molecular mechanism responsible for the biphasic response in this test is still debated, it has been speculated that it may be due to activation of the nociceptin/orphanin-FQ (NOP; ORL1, opioid receptor like) receptor which compromises buprenorphine's antinociceptive effect (reviewed in Cowan and Lewis, 1995 and Budd and Raffa, 2005). Part of the support for this view is that nociceptin/orphanin-FQ, the endogenous ligand for the NOP (ORL1) receptor, is pronociceptive in some pain models following i.c.v. (intracerebroventricular) dosing (Meunier et al., 1995; Reinscheid et al., 1995; Hara et al., 1997).

Regardless of the underlying molecular mechanism of the high-dose-related decline in buprenorphine effect, there is clearly a second component that antagonizes its antinociceptive action in some (but by no means all (Christoph et al., 2005)) preclinical tests (Ding and Raffa, 2009). It would be important to determine at least if the descending portion is similar in mammalian species. Such a demonstration would be consistent with the use of low-doses of buprenorphine for one clinical endpoint (analgesia) (Benedetti et al., 1998; Oifa et al., 2009) and high-doses for a different clinical endpoint (addiction medication (Boothby and Doering, 2007). Our aim here was to apply a novel approach to answer this question. Toward that end we here analyze data on mouse tail immersion from Raffa and Ding (2007), as well as new data (Cowan, unpub-

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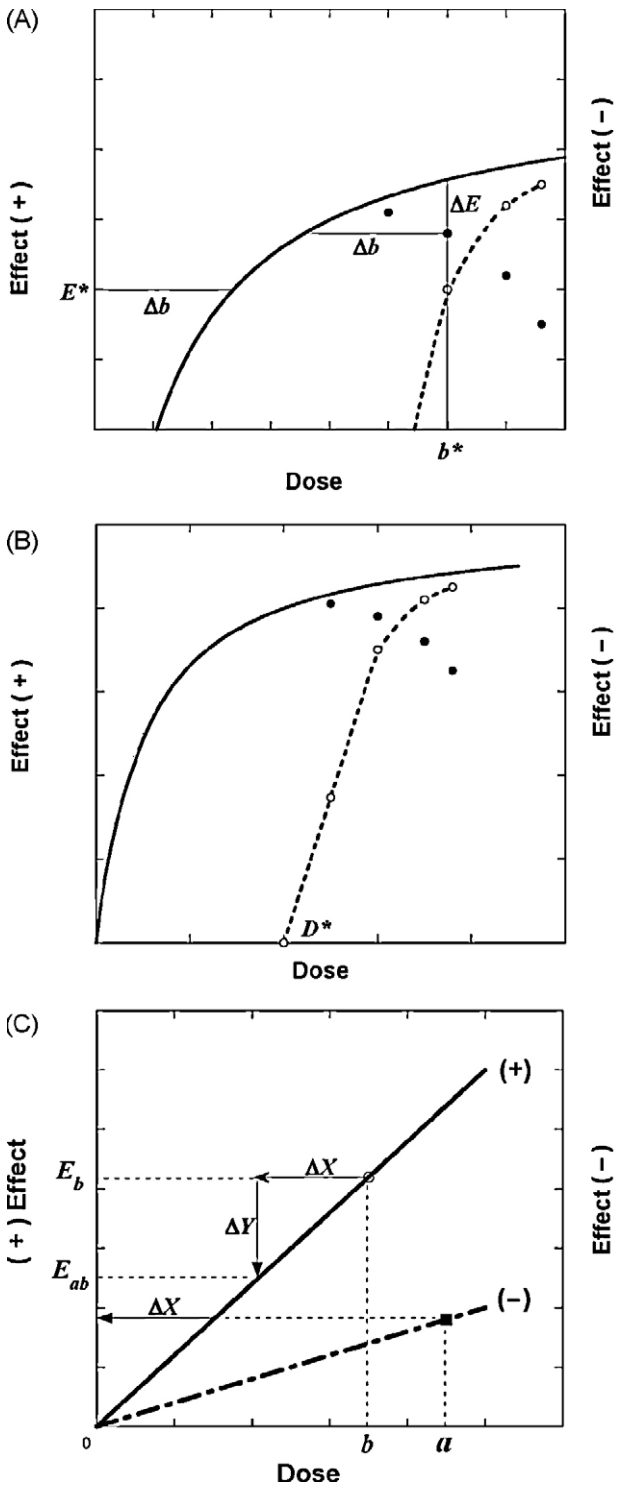

Fig. 1. (A) *Illustration*. A representative drop from the smooth curve (solid line), denoted ΔE, is equivalent to a decrease in agonist dose = Δb, a dose value on the smooth curve at effect E^* and, therefore, locates the point (b^*, E^*) for the (decreasing) second component of action (dotted line). (B) *Illustration*. A dose–effect curve (solid line) for doses up to dose D^* is monotone increasing, whereas for doses greater than D^* the effect decreases due to a second component of action. The dotted line, whose effect magnitudes are indicated on the right ordinate scale, represents decreasing effects for doses above D^* due to the second component of the drug's action. The combination of the decreasing and increasing components of action would yield the observed inverted-U values. (C) Illustration of two drugs that exert opposite effects. The upper line is an illustration of the dose–effect curve (not necessarily linear) of a drug whose effect is in a positive direction, whereas the lower line represents a drug that produces the opposite effect. The latter is plotted on the same coordinate system and, thus, its ordinate position denotes the magnitude of the negative effect.

lished results) in the rat, in a calculation that uses the concept of dose equivalence, the same principle that underlies additivity in isobolographic analysis.

2. Materials and methods

2.1. Experimental

The tail immersion/flick test data in mice reported in Raffa and Ding (2007) were used in the analysis. Briefly stated, the mice were placed into restraining holders and the distal portion of their tail was lowered into a temperature-controlled water bath (48 °C). The time interval between tail immersion and removal ('flick') of the tail was recorded as tail-flick latency. The cut-off time (to avoid injury) was 40 s. A baseline control latency value was obtained for each mouse before s.c. buprenorphine administration. After drug administration, the procedure was repeated and reaction times were compared to pre-drug reaction times. A similar procedure was followed in the rat tail immersion test, except that the water temperature was 50 °C and the cut-off time was 20 s.

2.2. Isobolographic analysis

The concept of dose equivalence is most familiar from isobolographic analysis of a combination of two drugs (reviewed in Tallarida, 2000, 2006, 2007) as briefly summarized here for two agonist drugs that exhibit a constant potency ratio. This means that for every level of effect the dose a of drug A alone, and the dose b of drug B alone, it follows that $a/b = R$, a constant. Most often the effect level analyzed is 50% of the maximum, from which it follows that $A_{50}/B_{50} = R$, where A_{50} and B_{50} are the unitary doses that give half-maximal effect. An arbitrary dose a that is less than A_{50} will require a dose b of B such that their sum = B_{50}. To calculate the quantity b, dose a is first converted to its B-equivalent, which is a/R. Thus, $b + a/R = B_{50}$, which can be rearranged to $b/B_{50} + a/RB_{50} = 1$ and, since $RB_{50} = A_{50}$, can be written in the more familiar form.

$$\frac{b}{B_{50}} + \frac{a}{A_{50}} = 1$$

The above equation, when plotted on an a–b Cartesian plot, is a straight line with intercepts A_{50} and B_{50}. All a–b combinations on this line, the isobole, represent pairs that are expected to yield the 50% effect. Because the dose-equivalent of drug A is *added* to dose b the condition derived is termed 'additive' and would demonstrate that there is no interaction between the agonist drugs. The concept of dose equivalence described here is employed, as shown in Section 3, in the analysis of the buprenorphine curve that arose from hot water tail immersion tests in the mouse (Raffa and Ding, 2007) and in the rat (Cowan, unpublished results).

3. Results

3.1. Theory

The concept of dose equivalence, described above, is used here in the analysis of the high-dose decreased effect that arises from the second component, when present, of buprenorphine's dose–effect curve. As illustrated in Fig. 1A, we see at some agonist dose b^* there is a decrease (ΔE) from the fitted curve. This decrease is associated with a decrease in agonist dose, Δb, a value that occurs at effect level E^* on the agonist's fitted curve. Thus (b^*, E^*) is a point on the dose–effect curve of the second component . In other words, b^* represents a dose at which the second component effectively nullifies a quantity (Δb) of the agonist's positive action that is sufficient to reduce its effect by the observed amount ΔE. Fig. 1B illustrates the result of analyzing the decreases in effect that constitute the inverted-U as described above. It is seen that this procedure allows the construction of the second component as a dose–effect relation in cases in which the interaction is additive.

A further illustration of the analysis for opposite effects is provided in Fig. 1C. A dose b of the '+' drug would produce the effect denoted E_b. The presence of the second drug in dose a produces an opposite ('−') effect and is equivalent to a dose reduction ΔX of the first drug. Accordingly the quantity ΔX is subtracted, thereby reducing its effect by amount ΔY and bringing it to the effect labeled E_{ab}. This process assumes that there is no interaction between the two drugs, *i.e.*, that the combination is simply additive. In this case the additivity is algebraic, *i.e.*, one subtracts the equivalent from