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Review Article

Are neuromuscular blocking agents being misused in laboratory pigs?

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Abstract

The literature (2012–4) describing experimental pig surgery was reviewed to estimate the extent to which neuromuscular block (NMB) is used, to examine methods for ensuring unconsciousness, and to identify the rationale for use of NMB and establish the anaesthetist's training. In the first stage of a two-stage review, NMB use was estimated using Web of Knowledge to identify articles describing NMB during pig surgeries. In the second stage, PubMed and Google Scholar were used to increase the number of articles for determining measures taken to prevent accidental awareness during general anaesthesia (AAGA). The corresponding authors of screened articles were emailed four times to establish the reason for using NMB and the anaesthetists' backgrounds (medical, veterinary, or technical). The first search revealed NMB use in 80 of 411 (20%) studies. Of the 153 articles analysed in the second stage, two described strategies to reduce AAGA. Some (6%) papers did not provide information on anaesthetic doses; citations supporting anaesthetic efficacy were found in only 13. Five of 69 papers using inhalation agents measured end-tidal anaesthetic concentrations based on human, not porcine, minimal alveolar concentrations. The methods in 13% of articles reporting anaesthetic depth assessment were incomplete or questionable, or both; four described using somatic motor reflexes. Corresponding authors of 121 articles reported that the principal reason for NMB was improved 'surgical visualization' (26%). Medical or veterinary anaesthetists supervised anaesthesia in 70% of studies; non-anaesthetists provided NMB, unsupervised, in 23. Nine respondents prioritized experimental expediency over pig welfare. In laboratory pig studies, AAGA may be prevalent; reported details of its attempted prevention are woefully inadequate.

Key words: anaesthesia; awareness; neuromuscular blockade; surgery; swine

Editor's key points

- Neuromuscular blocking agents (NMBs) are widely used in pig surgery for research.
- Inadequate monitoring of anaesthetic depth could result in accidental awareness.
- The authors undertook a literature review and author questionnaire about studies reporting pig surgery and NMB use.
- Results suggest accidental awareness is likely to be prevalent owing to inadequate monitoring and poorly trained staff.

The use of neuromuscular blocking agents (NMBs) in conditions of inadequate anaesthesia or analgesia in humans has resulted in accidental awareness during general anaesthesia (AAGA),[1] which may have severe psychological consequences.[2–5] The majority of affected humans report their experience of awareness verbally, but a proportion fail to report at all.[6] Estimating the extent of AAGA in infants and children is complicated by their inability to communicate effectively regarding awareness.[7] Difficulties are greater in animals because, unlike children, they never develop the capacity to articulate memories of AAGA that they may have experienced as infants.[4] To date, the challenge in determining whether animals receiving NMBs may have suffered AAGA has been avoided; it being assumed, presumably, that

there is universal compliance with the unsurprising recommendation that humane NMB use is absolutely dependent on the provision of adequate anaesthesia (Flecknell[8]; Hall and colleagues;[9] Tranquilli and colleagues[10]). Unfortunately, ensuring such provision is equally challenging, being based on 'signs' of anaesthesia that the veterinary anaesthetist assumes—without incontrovertible evidence—to be related to the cognitive, sensory, or emotional state of the animal.

Given the potential for severe effects on humans, a failure to assure unconsciousness in laboratory animals receiving NMBs constitutes a dereliction of the refinement principle (i.e. that experiments involving animals are conducted in a way that obviates their pain and suffering and optimizes their welfare). Refinement (along with replacement and reduction) forms an ethical defence for animal use in biomedical research (Burch[11]). Legislation restricts the use of NMB in animal experiments in many countries. In the UK, NMB can only be used in procedures licensed under the Animals (Scientific Procedures) Act 1986[12] [A (SP)A 1986] upon the granting of specific permission. Even in licensed procedures, the individuals administering NMB must be additionally and specifically licensed to do so on the basis of 'adequate training provided by competent research workers'. In the USA, the Institute of Laboratory Animal Resources requires that the proposed use of NMBs is carefully evaluated by the Institutional Animal Care and Use Committee (IACUC), 'to ensure the well-being of the animal'.[13] However, these measures *per se* provide no safeguards against AAGA; therefore, strategies have been proposed that attempt to reduce risk. These strategies include the following: (i) administering NMBs only once noxious stimulation demonstrably fails to elicit motor responses; (ii) (a) using an anaesthetic technique whose efficacy has been established in pilot studies on unparalysed animals[14] or (b) using a non-NMB-based anaesthetic whose efficacy has been critically and convincingly established in previous, though not necessarily related, studies involving animals of similar breed, sex, and age, undergoing similar procedures or (c) using end-tidal anaesthetic concentrations shown to produce adequate anaesthesia in minimal alveolar concentration (MAC) determination studies; or (iii) periodically allowing monitored neuromuscular block to wane to determine the presence or otherwise of spontaneous movement.[8] With each of these strategies, the possibility that the response of an individual animal to anaesthetic agents differs from that of test animals or that the technique of delivery may change (e.g. through equipment malfunction or human error) means that methods for monitoring the depth of anaesthesia in individual animals must be available.[8] Unfortunately, signs of anaesthetic depth in animals are poorly defined, and nociceptive responses are not always characterized by increases in arterial blood pressure and tachycardia.[15] Training and experience of the anaesthetist may overcome these difficulties; in the 5th National Audit Project (NAP5), AAGA was less likely when senior anaesthetists were involved,[5] and the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes[16] recommends that 'specialist advice' on anaesthesia should be obtained when these (NMB) agents are used.

Pigs are used extensively in biomedical research; 77 280 animals were used in the EU in 2011.[17] A proportion of these undergo surgical procedures that are associated with postoperative pain in human patients and in which profound muscle relaxation may be beneficial. It is more likely that NMB is used to achieve this in pigs compared with other laboratory animals, because NMB is widely recommended for tracheal intubation, which is relatively difficult in this species.[9 18] Two points are pertinent in considering AAGA in pigs: (i) individual pigs, like some human patients,[19] may not show sympathetic nervous signs when paralysed and exposed to noxious stimulation;[20] and (ii) bispectral index monitoring is unreliable in this species.[21 22]

In contrast to the A(SP)A 1986,[12] the transposition of the EU Directive 2010/63/EU[23] into UK law makes no specific statements about the use of NMBs in laboratory animals. This, and the publication of NAP5, prompted the present literature review. The aims of the review were as follows: (i) to estimate the extent to which NMB is used in pigs undergoing experimental surgical procedures; (ii) to determine what measures were taken to ensure that pigs were unconscious; (iii) to establish the reasons why NMB was felt to be necessary; and (iv) to identify the training background of the supervising anaesthetist.

Methods

A two-stage literature review was conducted on 24 and 25 July 2014. The first stage aimed to identify the extent of NMB use and used the search engine Web of Knowledge (https://webofknowledge.com) and search criteria for articles describing pig surgery (full search queries are shown in Fig. 1) published between 2012 and 2014. Only papers available through the University of Edinburgh (25 000 journals) were used. Identified articles underwent further analysis only if they described: (i) the anaesthetic technique used; and (ii) a surgical procedure that is recognizably painful in human subjects, in other words that it has supporting citations from the medical literature or has been recognized as potentially painful in other animal species by previous reviewers[24] or by the American College of Laboratory Animal Medicine (ACLAM) Analgesic Task Force (2007).[25] Consequently, only articles describing skin incision,[26] skin burning,[27] craniotomies,[28] laparotomies,[29] laparoscopies,[30] orthopaedics,[31] thoracotomies,[32] and transluminal endoscopy[33] were examined. Studies were excluded if they: (i) described the use of fewer than three pigs; or (ii) were pharmacological assessments of NMB drugs. The materials and methods sections of the article were read (by A.G.B.) to determine whether NMB had been used or not. Articles were categorized as 'NMB use described' or 'NMB use not described'. The country of origin of the article was recorded for the first-stage sample.

The second stage aimed to increase the sample size available for further analysis and used Web of Knowledge, Pubmed (http://www.ncbi.nlm.nih.gov/pubmed), and Google Scholar (http://scholar.google.co.uk/). The keywords used were 'pig' and the names of specific NMB agents described in papers identified in the first stage. Resulting articles describing the same operations were then filtered using the same aforementioned inclusion and exclusion criteria. Articles from both stages were then combined and the methods sections examined (by A.G.B.) to identify evidence of concerns with AAGA and to record specific measures described to minimize its likelihood. Consequently, the presence (or absence) of the following were recorded: (i) the description of specific strategies (see this page, first column, paragraph 2) taken to reduce the risk of AAGA; (ii) details (drugs, doses, and dosing intervals) of the anaesthetic technique, with citations (including MAC values) supporting its efficacy; and (iii) details of the methods used to determine anaesthetic depth. Descriptions of depth of anaesthesia monitoring were recorded as being absent or present. These descriptions were examined to determine the variables used to assess depth of anaesthesia, the frequency of assessment, and the training and experience of the assessor.

The total number of pigs (mean and range) involved in articles describing NMB use was calculated.