Commentary

Hypoxia, hyperoxia and breathing

1. Introduction

Between 1960 and 1980, I had the privilege of collaborating with two of the most remarkable French respiratory physiologists, initially with Pierre Dejours and subsequently with the late Roland Lefrançois. Both were interested in the regulation of breathing. It is well known that the major aim of regulation of breathing is to maintain the oxygen pressure and the acid-base balance in the arterial blood as constant as possible, especially when the energy expenditure increases (as during muscular exercise) or when the characteristics of the inspired gases change (as with hypoxia due to high altitude exposure). In order to achieve efficient regulation, the muscles of the thorax (also acting as a respiratory pump) are stimulated by the central (brain) respiratory areas, the latter themselves being continuously informed about the chemical composition of the arterial blood by specialized structures, the arterial chemoreceptors. In the early sixties, both Dejours and Lefrançois were concerned with the contribution of the arterial chemoreceptors to the regulation of breathing in humans at rest and during muscular exercise.

Here, I would like to review briefly their contributions to the understanding of respiratory control. These contributions came by way of carrying out experiments, on adult animals and humans, that were designed to change the partial pressure of oxygen in the inspired air (PIO_2) presented to them either as hyperoxia or as hypoxia. I would like to consider this attempt as my tribute to their teaching and its influence not only on my research, but also of a whole generation of respiratory physiologists all over the world.

In 1960, Dejours, already a well-known respiratory physiologist (having had worked in the fifties with stalwarts like Wallace O Fenn and Herman Rahn in the USA), was the head of a large laboratory in Paris where many fellows from France and overseas trained. I joined his laboratory as a young post-doc and my job during those years consisted essentially of serving as a naïve subject for his experimental studies. Additionally, I had to make measurements of ventilation; these were rather laborious as the recordings then were made on large sheets of paper. Moreover, in those days, the subject under study had to wear a tight-fitting facemask (or a mouthpiece), which was fairly uncomfortable in itself. Besides, the investigation lasted several hours; further distress was added by the presence of several large tubings and valves all around that were required for the collection of expired gases. The gases were then fed into a large spirometer which was almost a metre high as it had to have a capacity of several hundreds litres. The chemical analysis of the expired gases was performed by a painstaking procedure using a Scholander apparatus . The motion of the spirometer drum was mechanically registered on sheets of paper, allowing us to calculate the volume of expired gases as a function of time. Dejours insisted that the volume of the expired gases and the ventilation values to be calculated, be carried out *cycle by cycle*. Later on, this turned out to be a rather bright idea as it led us to the study of 'transitory phases'. The latter concept is best explained as an abrupt change in ventilation at the onset and at the end of muscular exercise, i.e within a few seconds or within one or two respiratory cycles (as also when ventilation changes rapidly when pure oxygen is breathed instead of ambient air). But for us this meant having to pore over metres and metres of recording paper and looking at hundreds of breathing cycles; each, having a duration and a height of a few millimetres (on paper), had to be measured precisely. Dejours got not one of us, but two of us fellows or laboratory assistants to carry it out in duplicate, so there was no way that we could take liberties with these measurements!

Indeed, in those days, there was no electronic equipment to record breathing or even calculators to compute means and standard deviations. As a matter of fact, Lefrançois who had joined the laboratory a few years earlier on, started in the following years to use the Fleisch pneumotachograph that allows

recording of respiratory airflows. And, to my knowledge, he was the first in France to use a cumbersome electronic integrator, notwithstanding a continuous drift that was impossible to control, thus leaving us to calibrate it repeatedly. Nevertheless, this was an advance, as it allowed us to compute the volume of air going in and out of the lungs from airflow values. Lefrançois was a passionate investigator and I was fortunate, and have been forever grateful to him, for having swept me into high altitude studies. He invited me to join him during all eight sojourns of investigations that he led to the Andean mountains between the years 1964 and 1973. During our studies carried out in Bolivia at altitudes ranging between 2,700 and 5,200 meters, we showed that the control of breathing of subjects born and living permanently at high altitude (natives) was markedly different from that of subjects recently acclimatized to altitude (sojourners)(see below). That was a relatively new finding then and repeatedly confirmed in the following years by other investigators.

But, after more than 40 years of teaching and research in the respiratory field, I am astonished by the fact that the interpretation of the contribution of hypoxia to the regulation of breathing is still evolving. Of course, it is unquestionable that the major effect of hypoxia is to provoke, after a short latency, a compensatory increase in ventilation. Here, as I will briefly review, the mechanism being considered appears more complex than postulated 40 years ago. Interestingly, this is not because technology has recently provided the investigator with new tools to carry out experiments, but more because observations are being made more thoroughly, particularly in humans and in unanaesthetized and free-moving animals.

2. Ventilatory response to hyperoxia: the oxygen test

By the late fifties, the role of the chemoreceptors in the regulation of breathing was fairly well known. This had been made possible by being able to record the afferent activity of the carotid sinus and aortic nerves in several animal species, and being able to use the techniques of electrical stimulation of the cut end of nerves and the influence of denervation studies. For obvious reasons, these experiments can not be performed on humans, but since they too had the same chemo-sensitive structures (confirmed from histological studies) and present similar functional responses to respiratory stimuli (hypoxia and, or hypercapnia) as seen in animals in the laboratory, it was likely that in human beings, the part played by the reflexogenic drive to breathing was the same as in animals.

Dejours asked the following question: If a ventilatory oxygen drive does exist in humans under a given condition, how could it be demonstrated? The inhalation of a high oxygen mixture will suppress the drive, and it is expected that ventilation would decrease in response. Conversely, the inhalation of a hypoxic mixture will enhance the drive and an increase in ventilation should be anticipated (see Dejours 1962). In practice, most physiologists have studied the expected changes in ventilation following a few minutes' inhalation of either a hyperoxic or a hypoxic gas mixture. Actually, at rest and at sea level, the ventilatory response to hyperoxic mixtures appears quite variable but it seems definitely related to the duration of the exposure to hyperoxia. Indeed, a brief review of the literature shows that when a high oxygen mixture is breathed (instead of ambient air) at sea level for 4 to 20 min, ventilation does not change or may increase slightly. Whereas, after a few breaths of pure oxygen or after 1 or 2 min of breathing it, a significant reduction in ventilation is observed (Gautier 2003).

From similar observations, Dejours realized that the method used for evaluating the oxygen-led ventilatory drive, which consisted of studying the effects on ventilation of inhalation of oxygen mixture of high concentrations *only after* several minutes of such an inhalation, may have been inadequate. Actually, a prolonged oxygen inhalation not only suppresses the peripheral oxygen drive, but can also provoke several other (supposedly) central chain of events, that may ultimately also influence ventilation. According to Dejours's proposal (see below), it is possible to investigate a change in the oxygen drive without it being influenced by secondary reactions. Thus, under a given condition, if there is a noteworthy chemoreflex drive, the inhalation of a high oxygen mixture will increase the alveolar PO_2 with the first breath, and within a few seconds when (arterial) blood with higher PO_2 from the lungs perfuses the peripheral arterial chemoreceptors, their activity should be expected to decrease. Therefore, at this time, it should be possible to observe a decrease in ventilation. With such a method, the influence of most of the secondary factors can be overcome (as that will show up only after a delay or a certain

latency). The use of oxygen inhalation can be restricted to one or two breaths (often referred to as the oxygen or the 'Dejours' test) or can be prolonged for several breaths.

Using the oxygen test technique, Dejours observed that at rest and at sea level, ventilation decreases *transiently*, by about 10% after a delay of 10 s, that is, corresponding to the circulation time between the lung and the carotid chemoreceptors. If at first the subject was to be made hyperoxic by continuously breathing a mixture containing 33% oxygen, resulting in an alveolar PO_2 of about 170 mmHg, the inhalation thereafter of 100% oxygen for one more breath, is not seen to be accompanied by any significant change in ventilation. This observation shows that the alveolar PO_2 threshold for the stimulation of chemoreceptors by oxygen, lies somewhere between 100 and 170 mmHg. This conclusion agrees well with the data obtained by direct recordings of chemoreceptor activity (afferent activity) in animals as a function of PaO_2 (partial pressure of arterial oxygen).

The oxygen test has been confirmed and used by Dejours and his collaborators in various situations, such as in people at rest, during muscular exercise at sea level and during short-term acclimatization to high altitude. Using also the oxygen test at altitude, we have confirmed the results of Dejours and, in addition, we have shown that the ventilatory oxygen drive is much smaller in subjects born and living permanently at altitude. We also confirmed that after a few minutes of 100% oxygen breathing, the ventilation was not significantly affected as compared to that by (normoxic) ambient air (Lefrançois *et al* 1968).

The above results clearly indicate that the ventilatory response to hyperoxia lasting several minutes presents a *biphasic time-course*. Whereas the *initial* decrease in ventilation has been, according to Dejours's studies, satisfactorily attributed to a sudden reduction in peripheral arterial chemoreceptor drive, the mechanisms involved in the subsequent increase or *recovery* in ventilation toward or exceeding control normoxic values have not been, to our knowledge, clearly identified. This is probably because, for technical reasons, studies can be carried out only in humans. Indeed, the oxygen test is difficult to perform in awake animals. Under anaesthesia, the effects of short (a few seconds) or long term (a few minutes) administration of oxygen on ventilation are not easy to interpret: the results seem to be related to the depth of anaesthesia which is often difficult to assess, or maintain at a constant level, in experimental studies (Gautier *et al* 1986). However, in *awake* cats and dogs with the carotid body surgically denervated, which is the main source of reflex ventilatory stimulation under hypoxic conditions, inhalation of oxygen instead of ambient air produces, within a few minutes, a significant increase in ventilation which may then increase more than what is seen in intact animals.

We have proposed that this oxygen-related hyperventilation seems to be of the same nature as the secondary augmentation of ventilation, which follows the transient depression observed during prolonged inhalation of oxygen in human beings. In other words, the carotid denervation unmasks a *late* originating stimulating effect of oxygen on respiratory centres, which in intact laboratory animals and humans is partly offset by the *early* depressing effect of oxygen on peripheral chemoreceptor drive. It follows that in the absence of the peripheral chemoreceptors input, ventilation seems to be rather dependent on the level of oxygenation of the central respiratory networks themselves. Indeed, in carotid-denervated as compared to intact animals, it has consistently been observed that ventilation is decreased during room air breathing; it is increased during oxygen breathing as seen above and conversely, during mild hypoxia, ventilation is significantly depressed.

3. Ventilatory response to hypoxia

The fact that the ventilatory response to hyperoxia as described more than 40 years ago and reviewed above presents a biphasic pattern, should be kept in mind as more recent studies, also carried out initially in humans, have shown that the ventilatory response to hypoxia appears to show a biphasic time-course (see Bisgard and Neubauer 1995). Indeed, upon a sudden exposure to hypoxia, ventilation increases abruptly in about 3–5 min, then it declines progressively and after 20–30 min of hypoxia, the initial increment in ventilation may be reduced by more than 50%. It thus appears that the time-course of the ventilatory response to hypoxia is the mirror image of the response to hyperoxia. The time-dependent fall in ventilation which has been termed *ventilatory roll-off* or *hypoxic ventilatory decline* has also been reported in a few animal species. The possibility that this response is due to adaptation of the arterial chemoreceptors, has been refuted by experimental studies suggesting that the observed decline

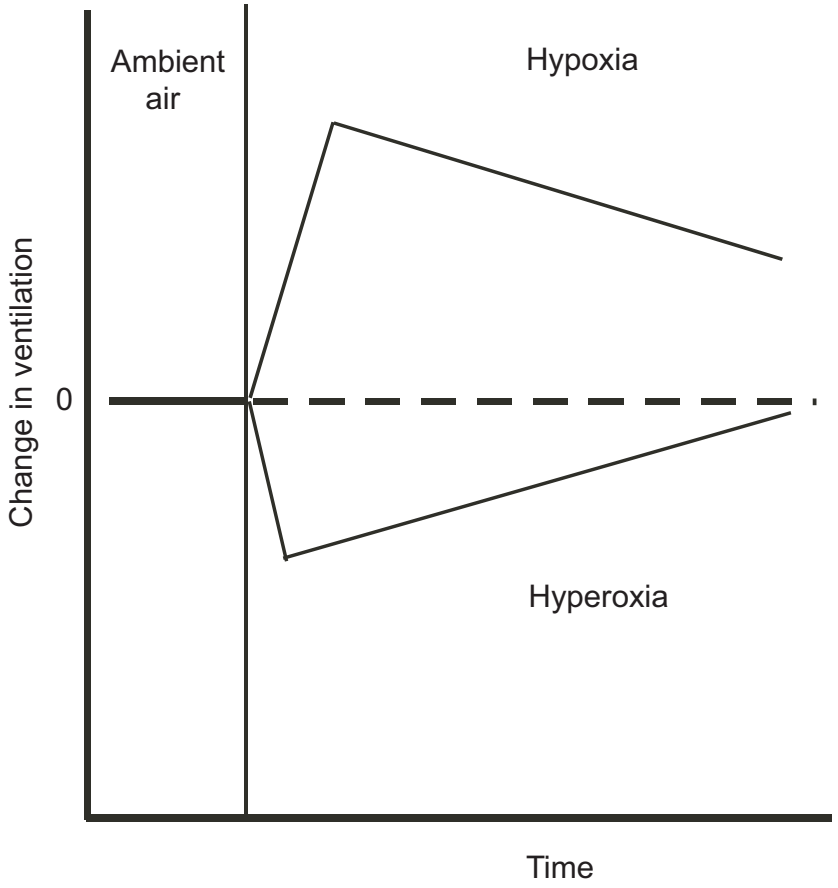

Figure 1. Schematic representation of the effect on ventilation of breathing hypoxic or hyperoxic gas mixtures as compared to ventilation in ambient air (normoxic conditions). It may be noted that the change in both cases is biphasic (see text for explanation; an increase is represented upwards and a decrease, downwards). No scale has been accorded either to ventilation change or to the course of time over which it occurs, as their actual values are related to the degree of hypoxia or hyperoxia used.

results from a direct effect of hypoxia on the brain (central depression). The recovery of the initial ventilatory response to hypoxia, which had declined during the 20–30 min of exposure to hypoxia, is examined below in § 4. Finally, it appears that during exposure to hypoxia, the ultimate respiratory drive reflects a balance between the stimulation of the peripheral chemoreceptors and the central depression of hypoxia on ventilation. Conversely, during exposure to hyperoxia, as described above, the ventilatory activity results from a balance between the inhibition of the arterial chemoreceptors and the central respiratory stimulation. Neither component is constant because they express multiple events that may change at different rates.

During changes in arterial PO_2, the mechanisms involved in the response of the peripheral chemoreceptors are relatively well known, whereas the mechanisms implicated as *central effects* are not completely understood. The latter are probably not restricted to the brain stem and may affect supra-pontine structures. They are probably multifactorial in origin, and will not be reviewed here. Recent studies suggest that during sustained hypoxia, changes in the concentration of chemical neuromodulators or neurotransmitters in the central nervous system are likely to be an effective source (Bisgard and Neubauer 1995).

From the above considerations, it follows that the ventilatory response to hypoxia or hyperoxia may be nowadays more difficult to interpret than it was 40 years ago. It is widely admitted, that in addition to their classical, immediate effect on the arterial chemoreceptors, alterations in PO_2 may also, after a few minutes, act on the central nervous system directly. Therefore the duration of the exposure to the gas mixture becomes an important determinant of the response in quantitative measures. This is obviously the

case in humans in whom the ventilatory responses to hyperoxia or hypoxia that were described initially were varying. This is also seen in animals during experimental conditions, data from which is used as a rule to unravel the mechanisms involved in the responses to respiratory stimuli.

Furthermore, there are other factors, that are often neglected, and which may complicate the interpretation of results obtained from laboratory animals. First of all is the use of anaesthesia. This, as briefly indicated above, may seriously alter the response to respiratory stimuli in a non-predictive way. This explains why, in recent years, whenever possible, respiratory studies have been carried out on unanaesthetized animals and particularly in rodents. Secondly and more importantly, it has been well known for many years (and recently emphasized) that the exposure to hypoxia of young or small mammals such as rodents (and many other small species) provokes a decrease in thermogenesis and therefore a fall in body temperature. Both are related to the ambient temperature and result from a direct effect of hypoxia on the central nervous system. It follows that this hypoxic-hypometabolism must be taken into account in interpreting correctly the ventilatory response to a given hypoxic stimulation as this response results from complex interactions between excitatory and inhibitory influences on peripheral chemoreceptors, central respiratory neurons and metabolic pathways (Gautier 1996).

4. Respiratory plasticity

Respiratory plasticity is defined as a persistent change in the neural control system based on prior experience and is important in a wide range of adaptive responses. Several interesting examples which have been described or rediscovered recently in adult mammals will be reviewed briefly (Reeves and Gozal 2005).

It has long been established that *sustained hypoxia*, such as occurs with exposure to high altitude, will lead to time-dependent changes in control of breathing. For instance, the response to a subsequent acute hypoxic challenge is enhanced and furthermore, the ventilation is progressively increased during long-term exposure to hypoxia. This has been termed "ventilatory acclimatization", a phenomenon that has been well documented in a variety of mammalian species and particularly in humans. On the other hand, much less is known about the effects of *intermittent hypoxia*, which is defined as repeated episodes of hypoxia, interspersed with episodes of normoxia. The actual protocols used experimentally may vary greatly in cycle length and in the number of hypoxic episodes, but the compelling outcome is that these repeated episodes of hypoxia elicit persistent changes in a variety of physiological responses. It has been reported that intermittent hypoxia leads particularly, and not only in anaesthetized animals, to a long-lasting post-hypoxia facilitation of respiratory motor output. This has not been seen in normal awake or sleeping humans although it has been suggested that patients presenting with obstructive sleep apnoea, a typical example of intermittent hypoxia, exhibit hypoxia-induced facilitation when awake (Mitchell *et al* 2001; Neubauer 2001).

The last example of respiratory plasticity concerns the interesting and intriguing effects of *prior hyperoxia* on the ventilatory response to hypoxia. It has been shown that after the ventilatory response has declined during sustained hypoxia (roll-off described above), the recovery process to the initial abrupt response is slow under normoxic conditions (over 15 min) but this process is significantly accelerated by prior inhalation of 100% oxygen (7 min) (Easton *et al* 1988). In addition, it has also been shown in humans that a short hyperoxic exposure before isocapneic hypoxia (normal carbon dioxide levels) could potentiate the peak ventilatory response to hypoxia without modifying the late component of the biphasic ventilatory response (Honda *et al* 1996).

5. Conclusions

This short Commentary has been limited to a few of several studies. They clearly indicate that the mechanisms involved in the effects of hypoxia and hyperoxia on the ventilatory control and particularly on the *integrative* respiratory centres are far from being completely understood and are now believed to be more complex than what was postulated 40 years ago. Other mechanisms, which are beyond the scope of this short review, are currently the subjects of numerous studies which suggest that mediators or modulators are involved not only at the periphery but also at the level of the central nervous system. It must be

emphasized that all these recent studies do not contradict at all, but rather help to explain, the early findings of Pierre Dejours concerning the participation of the peripheral chemoreceptors in the regulation and control of breathing.

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