The following article is an abridged version of a paper by Dampney and Horiuchi (in *Progress in Neurobiology*, 71: 359-384) and is for the purpose of private study only.
1. INTRODUCTION

The ultimate goal of studies on the functional organisation of central pathways that control the cardiovascular system is to elucidate how such pathways operate in intact conscious animals and humans, under both normal and pathological conditions. In recent years many studies have addressed this question, building upon the many advances that have resulted from anatomic studies as well as physiological and pharmacological studies in anaesthetised animals (for reviews of these earlier studies see Guyenet, 1990; Dampney, 1994; Blessing, 1997). One of the most useful approaches that has been used in studies on central cardiovascular control mechanisms in conscious animals has been the application of methods based on the expression of immediate early genes, particularly the c-fos gene, to identify central neurons that are activated by a specific stimulus. This method, when used alone or in combination with other methods, has yielded much new information on the functional organisation of central cardiovascular pathways as they operate in the conscious state.

2. THE METHOD OF c-fos EXPRESSION AS A MARKER OF NEURONAL ACTIVATION: ADVANTAGES AND LIMITATIONS

The immediate early genes are a family of genes whose transcription is activated rapidly and transiently within minutes following the application of a stimulus (for reviews see Morgan and Curran, 1989; Sheng and Greenberg, 1990; Morgan and Curran, 1991). In the case of the immediate early gene c-fos, its transcription leads to the production of the protein Fos, which then combines with Jun, the protein product of another immediate early gene to form a heterodimeric transcription factor that regulates the expression of so-called late response genes (Fig. 1). In nerve cells, the stimulus inducing immediate early gene expression may be synaptic excitation, a hormone or other stimulus that causes neuronal depolarisation, while the protein products of the late-response genes are believed to subserve longer-term responses of the neuron to the stimulus (e.g. altered production of a neurotransmitter or receptor) (Sheng and Greenberg, 1990; Morgan and Curran, 1991). Synaptic inhibition, however, does not appear to result in c-fos expression (Li and Dampney, 1994).

Studies in the late 1980s demonstrated that c-fos expression (detected by labelling of the Fos protein product or by in situ hybridization to detect c-fos mRNA) could be used as an effective marker of activation of brain neurons in response to specific stimulation (Dragunow and Robertson, 1987; Hunt et al., 1987; Morgan et al., 1987). The production of Fos is accompanied by the production of Fos-related antigens, which are also believed to regulate transcription (Morgan and Curran, 1991). Because many of the studies discussed below used antibodies that recognised both Fos-related antigens as well as Fos itself, the term “Fos-like immunoreactive (Fos-LI)” will be used in this review to refer to neurons that are labelled by this method.

The advantages and limitations of the method of c-fos expression as a marker of neuronal activation has been discussed in detail previously (Dragunow and Faull, 1989; Krukoff, 1993; Rowland, 1998). In brief, the major advantages are that (1) the method allows individual activated neurons to be identified, in contrast to earlier functional mapping procedures which relied on the uptake of 2-deoxy-D-glucose as an index of metabolic activity; (2) when experiments are designed appropriately, populations of neurons activated by a specific defined stimulus in conscious animals can be identified, thus allowing a more complete picture of the effect of a stimulus on brain activation under normal conditions than is available from other methods such as single-unit recording; (3) under appropriate experimental conditions, the baseline level of c-fos expression is low, so that the signal to noise ratio is usually
relatively high; (4) the method can be combined with other procedures, such as retrograde or anterograde tracing or the immunohistochemical labelling of specific transmitter or transmitter-related enzymes, so that it is possible in many cases to determine the connections and chemical properties of activated neurons; (5) the method is at least semi-quantitative, so that it is possible to compare the effects of different experimental conditions on the number of activated neurons in particular brain region, provided that other factors which may influence the degree of c-fos expression are maintained constant. In particular, it should be noted that the antibodies that are used in different studies can differ significantly in terms of their sensitivity or their specificity for Fos and Fos-related antigens.  Furthermore, the degree of expression also depends upon the particular immunohistochemical procedure that is used.  Thus, when making quantitative comparisons between the degree of Fos expression under different experimental conditions, it is important that the same antibody and immunohistochemical procedure be used to ensure that the sensitivity of the labelling is the same under all conditions.

There are a number of other limitations which must also be taken into account when interpreting functional mapping data based on c-fos expression. First, neurons differ in their capacity to produce Fos. For example, neurons in the substantia nigra do not appear to produce Fos whatever stimulus is applied (Dragunow and Faull, 1989), while in somatic motoneurons (Herdegen et al., 1991) the onset of Fos production is considerably delayed (i.e. by several hours) compared with the period in most other neurons (typically about 30-60 min). Second, strong and sustained stimulation of neurons is usually required before c-fos expression occurs (Dragunow and Faull, 1989; Li and Dampney, 1994). Thus, the absence of Fos-LI neurons in a particular region following a stimulus does not necessarily mean that no neurons in that region were activated by that specific stimulus. Despite this limitation, a study which compared Fos as a marker of neuronal activation with several other immediate early gene-encoded proteins (Fos B, Jun B, Jun D, c-Jun and Krox-24) concluded that Fos was the most effective marker of neuronal activation under a variety of experimental conditions (Lanteri-Minet et al., 1994). Similarly, Chan et al. (1993) found that the expression of another immediate early gene (NGFI-B) occurred in only subsets of Fos-LI neurons in the hypothalamus of rats subjected to various stimuli. Thus, the available evidence indicates that c-fos, when compared with other immediate early genes, is the most useful general marker of neuronal activation.

Figure 1. Schematic diagram showing intracellular pathways leading to c-fos and c-jun expression, and the role of their protein products Fos and Jun, respectively, in regulating the expression of other genes (so-called “late-response” genes). Adapted from Morgan and Curran (1989), with permission.
Because a sustained stimulus (typically over a time period of 25 min or longer (Li and Dampney, 1994)) is usually required to evoke Fos production, it is often not clear whether Fos production evoked in neurons following a stimulus is a direct or indirect consequence of that stimulus. As an example, a sustained period of hypotension would unload baroreceptors and thus would be expected to reflexly increase sympathetic vasomotor and cardiac activity (and decrease cardiac vagal activity) via activation of neurons within the central baroreflex pathway. At the same time, hypotension will also cause the release of renin from the kidney and lead to increased levels of circulating angiotensin II (Reid et al., 1978), which in turn may lead to activation of central neurons via inputs from angiotensin II-sensitive neurons in brain circumventricular organs (McKinley et al., 1992; Ferguson and Bains, 1997; Potts et al., 1999). Furthermore, hypotension also leads to the release of vasopressin (Share, 1988) which is believed to modulate neurotransmission in the central baroreceptor reflex pathway (Cai et al., 1994).

It is well known that c-fos expression can be greatly affected by anaesthesia (Dragunow and Faull, 1989). For example, barbiturates may interfere with c-fos expression (Dragunow and Faull, 1989), whereas other anaesthetics such as urethane and chloralose cause a high level of baseline expression (Miura et al., 1994; Dampney et al., 1995; Rocha and Herbert, 1997). It is therefore preferable, whenever possible, that c-fos studies be performed in unanaesthetised animals. It has been suggested, however, that when anaesthesia is required, ketamine/xylazine is a suitable anaesthetic for studies of c-fos expression in neural pathways subserving cardiovascular regulation (Rocha and Herbert, 1997). Unless otherwise specified, all of the c-fos studies discussed in this review were performed on unanaesthetised animals.

3. OVERVIEW OF CENTRAL CARDIOVASCULAR PATHWAYS

Figure 2 is a schematic diagram which illustrates the main central nuclei that subserve the autonomic and neuroendocrine regulation of the cardiovascular system. Anatomical studies have shown that direct inputs to sympathetic preganglionic neurons in the thoracolumbar spinal cord mainly originate from five regions in the brain: the rostral ventrolateral medulla (RVLM), rostral ventromedial medulla, medullary raphe, A5 noradrenaline-synthesising cell group in the pons, and the paraventricular nucleus in the hypothalamus (PVN) (Strack et al., 1989). Although in their original study in the rat Strack et al. (1989) defined the rostral ventromedial medulla as the region just medial to the RVLM but lateral to the inferior olive, most authors do not make this distinction. Thus the term “RVLM” as used here refers to the region in the rostral ventral medulla that extends laterally from the lateral edge of the inferior olive.

![Schematic diagram showing the main nuclei in the brainstem and hypothalamus that regulate the sympathetic and vagal outflow to the cardiovascular system, and also the release of vasopressin from the pituitary. Abbreviations: IX, glossopharyngeal nerve; X, vagus nerve; IML, intermediolateral cell column; KF, Kölliker-Fuse nucleus; NA, nucleus ambiguus; NTS, nucleus tractus solitarius; PAG, periaqueductal grey; PVN, paraventricular nucleus; RVLM, rostral ventrolateral medulla; SON, supraoptic nucleus.](image-url)
The parasympathetic vagal outflow to the heart arises predominantly from the nucleus ambiguus in the medulla (Fig. 2). With regard to neuroendocrine regulation, vasopressin is the main pituitary hormone that regulates the circulation. This hormone is released as a consequence of activation of vasopressin-synthesising neurons in the magnocellular portion of the PVN and in the supraoptic nucleus (SON), whose axons project to the posterior pituitary (Fig. 2). On the input side, signals from a variety of peripheral receptors (e.g. baroreceptors, chemoreceptors, cardiopulmonary receptors, nociceptors etc), all of which can reflexly influence the autonomic and/or neuroendocrine outputs to the cardiovascular system, reach the central nervous system via spinal and cranial afferent nerves. Of particular importance in this regard is the nucleus tractus solitarius (NTS), which is the site of termination of primary afferents from a variety of visceral receptors that reflexly affect cardiovascular function (Spyer, 1990). In addition, although not shown in Fig. 2, there are also inputs originating from cortical and subcortical centres that evoke autonomic and neuroendocrine effects in response to alerting or emotional stimuli (Blessing, 1997). All the nuclei depicted in Fig. 2 are involved in mediating responses activated by some or all of the various disturbances as discussed in the following sections.

4. EXAMPLES OF c-fos EXPRESSION

Changes in arterial blood pressure, such as those resulting from postural changes or other cardiovascular disturbances, elicit a reflex autonomic response that acts to minimise the effect of the original disturbance and thus stabilise blood pressure. Thus, for example, a transient hypotension results in a reflex increase in vasomotor and cardiac sympathetic activity and a reflex inhibition of cardiac vagal activity, leading to an increase in vascular resistance, heart rate and cardiac output, thus restoring blood pressure back towards the original level. When the hypotension is sustained, there is also a reflex release of vasopressin from the pituitary (Thrasher et al., 2000).

![Figure 3.](image)

Figure 3. Upper panels: Drawings of hypothalamic coronal sections at the level of the paraventricular nucleus (PVN) and supraoptic nucleus (SON), showing the distribution of Fos-LI neurons following a control stimulus in a conscious rabbit, and of induced hypotension in another conscious rabbit. Each dot represents one Fos-LI neuron in each section. Lower panel: Photomicrograph showing dense Fos labelling in the SON following a period of hypotension. The field of this photomicrograph is indicated by the small rectangle bounded by the dashed lines in the section above. Other abbreviations: OT, optic tract. Modified from Li and Dampney (1994), with permission.
Several studies have used the method of c-fos expression to map systematically populations of neurons in the medulla and or supramedullary regions that are activated by a period of isovolemic hypertension or hypotension in the conscious rat (Chan and Sawchenko, 1994; Graham et al., 1995; Petrov et al., 1995; Tassorelli and Joseph, 1995; Chan and Sawchenko, 1998b; McLean et al., 1999; Grindstaff et al., 2000) or rabbit (Li and Dampney, 1992; 1994). In both species, hypertension and hypotension each induced a consistent and specific pattern of c-fos expression in the brainstem and forebrain, although the distribution patterns and the chemical characteristics of the Fos-LI neurons induced by these stimuli were significantly different from each other.

Isovolemic hypotension in conscious rabbits or rats (Li and Dampney, 1994; Graham et al., 1995) has been shown to evoke a high degree of c-fos expression in many medullary and supramedullary regions referred to above. For example, as shown in Fig. 3 above, this includes many neurons in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) in the hypothalamus. As mentioned above, these neurons synthesize vasopressin, which is then transported to the pituitary and released into the circulation in response to the hypotensive stimulus.

Similarly, in the medulla, hypotension evoked c-fos expression in many neurons in discrete regions, such as the RVLM (Fig. 4). These observations are consistent with many studies in anesthetized animals which have shown that these neurons are reflexly activated in response to a hypotensive stimulus, thus increasing sympathetic activity and restoring blood pressure.

![Image of c-fos expression in the medulla at the level of the RVLM.](image)

*Figure 4.* Example of c-fos expression in the medulla, at the level of the RVLM. The section on the upper left side shows very few Fos-LI neurons following a control stimulus in a conscious rabbit, whereas there are many Fos-LI neurons in the RVLM following a period of hypotension in another conscious rabbit. The high-power photomicrograph in the lower right panel shows two Fos-positive neurons in the RVLM (indicated by black staining in the nuclei) that are also immunoreactive for tyrosine hydroxylase (indicated by brown staining in the cytoplasm). Tyrosine hydroxylase is a catecholamine-synthesizing enzyme.
As mentioned above, immunohistochemical labelling for Fos can be combined with immunohistochemical labelling for other chemical markers, such as neurotransmitters or neurotransmitter-related enzymes. This is clearly shown in Fig. 4 above, which shows examples of two neurons labelled for both Fos (black staining in the cell nuclei) and tryosine hydroxylase (brown staining in the cytoplasm). Using this double-labelling procedure, it has been shown that about 70% of the neurons in the RVLM that are activated by hypotension are catecholamine-synthesizing neurons.

In conclusion, the phenomenon of c-fos expression has allowed us to learn much about the functional organisation of central cardiovascular pathways as they operate in the conscious animal. In addition, c-fos and other immediate early genes also appear to play a crucial role in modifying the functions of central cardiovascular neurons, leading to long-term changes in the regulation of the cardiovascular system both in normal and pathological conditions. Defining the specific long-term changes that occur in central cardiovascular pathways in conditions such as hypertension and heart failure, and the cellular mechanisms that underlie these changes, are major challenges for the future.

REFERENCES


